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THE CRANIAL MUSCLES OF TELEOSTS—2

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IN the previous paper, the cranial musculature of *Hilsa ilisha* (Ham), a member under the *Super family clupeoidea* was described while in the present one, three more forms under the same *Super family* are described and compared. Further descriptions on two types representing the *Super family elopoidae* are added.

FAMILY CLUPEIDAE *PELLONA FILIGERA* (Day)

The Mandibular Muscles

The *adductor mandibulae* consists of only a *mandibularis* and the *intramandibularis*, the *maxillaris* being absent.

The *mandibularis*. Fig. 9 (mnd)

Description: The muscle starts as a narrow piece and towards its insertion it becomes broad. There is a poorly developed ligament, lying anterior to the muscle, developed from the membrane lying in between the maxilla and the *mandibularis* which connects the bases of the maxilla and the mandible. It is not known whether this ligament is derived from the muscle or not.

Origin: It originates in a fleshy attachment from the preoperculum, symplectic and quadrate and runs obliquely downwards.

Insertion: On one hand the muscle is inserted by a fleshy base on the posterior part of the mandible and on the other it is continued as the *intramandibularis*.

Innervation: It is innervated by the mandibular branches of the N. *maxillomandibularis* V and N. *hyomandibularis* VII.

Action: It helps in the adduction of the mandible.

The *intramandibularis*. Fig. 9 (irmn)

Description: It is a broad piece of muscle triangular in shape.

Origin: It arises from the anterior end of the *mandibularis* and runs on the inner side of the mandible.

Insertion : It is inserted on the inner ventral border of the mandible.

Innervation : The mandibular branch of the N. *maxillomandibularis* V innervates the muscle.

Action : It assists the action of the *mandibularis*.

Comparison : In Hilsa the muscle arises from one of the anterior tendons of the *mandibularis* and here it arises from the fleshy anterior end of the *mandibularis* and it is short and broad.

The *constrictor dorsalis* is divided into the *levator arcus palatini* and the *dialator operculi*.

The *levator arcus palatini*. Fig. 9 (lap)

Description : The origin of the muscle is broad and towards its insertion it becomes narrow. It covers the anterior part of the *dialator operculi*.

Origin : It originates in a broad fleshy attachment from the frontal and the anterior part of the hyomandibula and descends to the pterygoid bones.

Insertion : It is inserted on one of the pterygoid bones without forming a tendon.

Innervation : The muscle is innervated by a branch of the N. *maxillomandibularis* V.

Comparison : In Hilsa it is a broad muscle while in this specimen the origin of the muscle is broad whereas towards insertion it becomes narrow.

The *dialator operculi*. Fig. 9 (do)

Description : It is a transverse piece of muscle lying dorsal to the *mandibularis*. Its anterior part is covered by the *levator arcus palatini*.

Origin : It originates in a fleshy attachment from the hyomandibula and the pro-otic.

Insertion : It is inserted on the dorsal part of the pre-operculum dorsal to the origin of the *mandibularis* without forming a tendon.

Innervation : This muscle is also innervated by the same branch of the nerve which innervates the *levator arcus palatini*.

Action : It assists in the elevation of the operculum.

The *intermandibularis*. Fig. 9, 10, (inmn & ih, ant, post)

Description : The muscle is fused with the *interhyoideus* to form a single muscle. It has two regions an anterior and a posterior. In the *i. anterior* the muscle fibres run longitudinally and converge to two points and in the *i. posterior* the fibres run longitudinally and spread on the outer side of the *i. anterior*.

Origin : The fused muscle originates in a fleshy attachment from the outer part of the ceratohyal and the bases of the third and fourth branchiostegal rays on each side. It runs forwards, meets its fellow from the other side to fuse together and the fused muscle gets separated into anterior and posterior regions.

Insertion : It is inserted on the bases of the two rami of the lower jaw.

Innervation : It is innervated by the mandibular branches of the N. *maxillomandibularis* V and N *hyomandibularis* VII.

Action : It helps in depressing the lower jaw.

Comparison : As in Hilsa the muscle fuses with the *interhyoideus* but the fused muscle has two regions an anterior and a posterior.

The Hyoid Muscles

All the hyoid muscles are similar to those of Hilsa.

Branchial Muscles

The *levator arcuum branchialium* are similar to those of Hilsa.

The *coracobranchialis*. Fig. 11 (cb, cbt)

Description : The muscle is a single broad sheet and it joins with its fellow from the other side in a narrow piece of muscle on the ventral side of the branchial arches (cbt).

Origin and insertion : As in Hilsa it originates from the pectoral girdle, runs forwards and is inserted on the basi-branchial of the fourth arch and besides having this insertion the anterior tips of the muscles from both the sides fuse together to form a narrow piece of muscle (cbt) below the fourth basi-branchial.

Innervation : It is innervated by a branch of the post-trematic nerve of the Xth.

Action : It helps in lowering the ventral parts of the branchial arches.

Comparison : Unlike in Hilsa in this specimen the two *coracobrachialis* meet on the ventral side of the head below the basi-branchial of the fourth branchial arches.

The *subarcuales recti* are not developed in any of the branchial arches except in the fifth where they occur as the *subarcuales communis* which are very poorly developed.

The *transversi ventrales*. Fig. 2 (tv 3 to 5)

Description : These muscles are developed in the last three branchial arches only and in the first two they are absent. In the third branchial segment it is not well developed while in the last two they are thin and narrow.

Origin and insertion : They originate from the corresponding hypobranchials, run inwards and meet their fellows from the opposite side and fuse to form complete sheets of muscles lying in between the hypobranchials of the third to fifth branchial arches. Both origin as well as insertion of the muscles are fleshy.

Innervation : The muscles are innervated by the post-trematic nerves of the IXth and Xth.

Action : They also assist the other muscles in drawing the ventral parts of the branchial arches down.

Comparison : Unlike in Hilsa in this specimen the muscles are developed in three segments and they are very narrow.

The *cucullaris* is not developed as in Hilsa.

The *sphincter oesophagi*. Same as in Hilsa.

All other muscles are similar to those of Hilsa.

CHATOESSUS NASUS

The Mandibular Muscles

The *adductor mandibulae* is separated into a *mandibularis* and the *intramandibularis*.

The *mandibularis* (Fig. 12 mnd) and the *intramandibularis* (Fig. 12 irmn) are similar to those of Pellona. So also the *Levator arcus palatini*.

The *dialator operculi*. Fig. 12 (do.)

Description : It is triangular in shape, broad at its origin and narrow towards its insertion. Both origin and insertion of the muscle are fleshy. It lies behind the *levator arcus palatini*.

Origin and insertion : It arises in a broad fleshy attachment from the frontal pterotic runs vertically downwards and is inserted on the dorsal part of the pre-operculum.

Innervation : It is innervated by a branch of the N. *maxillomandibularis V*.

Action : It assists in the elevation of the operculum.

All other muscles are similar to those of Hilsa.

FAMILY ENGRAULIDAE

COILIA DUSSUMIERI (C. V)

The Mandibular Muscles

The *adductor mandibulae* consists of only a *mandibularis* and the *intramandibularis* as in Hilsa. The *maxillaris* is absent.

The *mandibularis*. Fig 13 (mnd.)

Description : It is a broad sheet of muscle. The origin of the muscle is fleshy and towards the insertion it gets separated into two, one part resolves into a tendon (tn) while the other part remains fleshy. The dorsal part of it is covered by the *levator arcus palatini* and the *dialator operculi*.

Origin : It originates in a fleshy attachment from the ventral part of the pro-otic, hyomandibula, the symplectic, the whole length of the pre-operculum and the quadrate and it runs vertically downwards.

Insertion : The tendinous part of the muscle is continued as the *intramandibularis* and the fleshy part is inserted on the inner ventral posterior part of the mandible.

Innervation : It is innervated by the mandibular branches of the N. *maxillomandibularis V* and the N. *hyomandibularis VII*.

Action : It helps in the adduction of the mandible.

Comparison : Unlike in Hilsa there is only one tendon arising from the anterior ventral tip of the muscle.

The *intramandibularis*. Fig. 13 (irmn.) is similar to that of Hilsa.

The *constrictor dorsalis*. It is divided into the *levator arcus palatini* and the *dialator operculi*.

The *levator arcus palatini*. Fig. 13 (lap.)

Description : It is a long narrow muscle lying dorsal to the *mandibularis* covering a part of the *mandibularis* on its dorsal side.

Origin and insertion : It originates in a fleshy attachment from the frontal and descends to the pro-otic and is inserted on this bone without forming a tendon.

Innervation : It is innervated by a branch of the N. *maxillomandibularis* V.

Action : It fills the concavities of the bones as in the case of Hilsa.

Comparison : Unlike in Hilsa in this specimen the muscle is a narrow piece.

The dialator operculi. Fig.13 (do.)

Description : It is a narrow piece of muscle lying posterior to the *levator arcus palatini*.

Origin and insertion : It arises from the hyomandibula dorsal to the origin of the *mandibularis* in a fleshy attachment runs backwards and is inserted on the dorsal side of the pre-operculum.

Innervation : This is also innervated by the same nerve which innervates the *levator arcus palatini*.

Action : It helps in the elevation of the operculum.

Comparison : The muscle is very narrow unlike in Hilsa.

All other muscles are similar to those of Hilsa.

SUPER FAMILY ELOPOIDAE

FAMILY MEGALOPIDAE

MEGALOPS CYPRINOIDES

The Mandibular Muscles

The *adductor mandibulae* is separated into a *maxillo-mandibularis* and an *intramandibularis*.

The *maxillo-mandibularis*. Fig. 14 (mxmnd.)

Description : The *maxillaris* and *mandibularis* are fused together. The origin of the muscle is fleshy and the insertion is tendinous.

Origin : It originates as a fan-shaped muscle chiefly from the pre-operculum, symplectic and quadrate and runs forwards. The rest of the origin of the muscle which is from the posterior part of the mandible is reduced to a tendon (tnr).

Insertion: Two tendons (tn and tn₂) arise from the anterior part of the muscle. Of these the former arises from the dorsal anterior part and the latter arises from the ventral inner part of the muscle. The dorsal tendon (tn) is inserted on the base of the maxilla and the ventral tendon (tn₂) is continued as the *intra-mandibularis*. The tendon (tn₁) joins with the main tendon of the muscle (tn).

Innervation: The muscle is innervated by the *maxillaris* and mandibular branches of the N. *maxillomandibularis* V and the mandibular branch of the N. *hyomandibularis* VII.

Action: It assists in pushing the ventral part of the maxilla forwards.

Comparison: Unlike in Hilsa, here a *maxillomandibularis* is developed.

The *intramandibularis*. Fig. 14 (irmn). Same as in Hilsa.

The *levator arcus palatini* and the *dialator operculi* of the *constrictor dorsalis* are similar to those of Hilsa in all respects except that here, the ventral part of the *levator arcus palatini* is covered by the dorsal part of the *maxillomandibularis*.

The *intermandibularis*. Fig. 14 (inmn & ih).

The muscle is similar to that of Hilsa.

All the hyoid muscles are similar to those of Hilsa.

The Branchial Muscles

The *levatores arcuum branchialium*. Fig. 15 (labe).

Description: They are five in number. The first four closely adhere together so that they appear as one single sheet of muscle as in Hilsa. The fifth remains separate.

Origin and insertion: All of them originate from the parasphenoid and the first four are inserted on the corresponding epibranchials and the fifth gets merged with the membrane which connects the pectoral girdle with the branchial arches.

Innervation: The muscles are innervated by the post-trematic branches of the IXth and Xth nerves.

Action: They assist in the elevation of the branchial arches.

Comparison: As the first four muscles lie close together they appear as one sheet as in Hilsa.

The *coracobranchialis*. Fig. 16 (cbe, cbi).

Description: The muscle is separated into *c. externus* and *c. internus*. Both origin and insertion of the muscles are fleshy.

Origin and insertion: They originate from the pectoral girdle and are inserted on the fifth branchial arch.

Innervation: The two parts are innervated by the post-trematic nerve of the Xth.

Action: They assist in the retraction of the branchial arches.

Comparison: Unlike in Hilsa, here, it is separated into *c. externus* and *c. internus*.

All the remaining muscles are similar to those of Hilsa.

FAMILY SYNODIDAE

HARPODON NEHREUS, (HAM)

The Mandibular Muscles

The *adductor mandibulae*

In this specimen it consists of the *maxillaris*, the *mandibularis* and the *intramandibularis*.

The *maxillaris*. Fig. (17 mx).

Description: It is a transverse piece of muscle covering the dorsal half of the *mandibularis*. The origin and the insertion of the muscle are fleshy.

Origin and insertion: It originates only from the middle part of the pre-operculum and runs forward to be inserted on the middle part of the maxilla.

Innervation: It is innervated by the maxillary branch of the N. *maxillomandibularis* V.

Action: It helps in opening the mouth.

Comparison: It is well-developed.

The *mandibularis*. Fig. 17 (mnd. ant; mnd. post.)

Description: It is a thick piece of muscle separated into two parts of which one lies anterior to the pre-operculum and the other posterior to the pre-operculum.

The origin of both the muscles are fleshy and tendinous towards their insertion.

Origin: The anterior part originates from the frontal pro-otic sub-orbitals, hyomandibula, symplectic, pre-operculum and the quadrate and the posterior from the frontal.

Insertion: Both the parts run vertically down with the anterior part resolving into a tendon (tn) which divides into two (tn1 & tn2). One of these tendons (tn2) is inserted on the posterior inner part of the mandible and the other is continued as the *intramandibularis*. The posterior part also resolves into a couple of tendons (tn3 & tn4) which are inserted on the posterior part of the mandible.

Innervation: They are innervated by the mandibular branches of the N. *maxillomandibularis* V and the N. *hyomandibularis* VII.

Action: They assist in the elevation of the lower jaw.

Comparison: In this specimen the muscle is separated into two by the operculum which is not found in any other specimen examined and the anterior originates from the suborbitals beside the origin from the other bones and the posterior takes its origin from the frontal.

The *intramandibularis*. Fig. 17 (irmn.)

Description: It is similar to that of Hilsa, in all respects except for a separate tendinous origin from the pre-operculum and it originates from the tendon of the *mandibularis*.

The *constrictor dorsalis* has only the *levator arcus palatini*, the *dialator operculi* is absent.

The *levator arcus palatini*. Fig. 18 (lap.)

Description: The muscle lies hidden below the *mandibularis*. It originates in a tendon and the insertion is fleshy.

Origin and insertion: The tendinous origin of the muscle is from the frontal; runs vertically downwards and is inserted on the palato-quadrate bar.

Innervation: It is innervated by a branch of the N. *maxillomandibularis* V.

Action: As it has an insertion of the palato-quadrate bar, it indirectly helps in the opening of the mouth.

Comparison: Unlike in any other specimen examined, the muscle extends upto the palato-quadrate bar and gains an insertion on it.

The *dialator operculi* is not developed.

The *intermandibularis*. Fig. 19 (inmn & ih.)

Description: The muscle is fused with the *interhyoideus*. In the anterior region some of the muscle fibres run longitudinally and converge to two points forming a spindle-shaped (sp) region in the muscle. The other muscle fibres from the posterior region run forwards and spread over the spindle-shaped region of the muscle.

Origin and insertion: The fused muscle from each side originates from the posterior part of the ceratohyal and the base of the fifth branchiostegal ray and runs forwards covering the anterior parts of the first four branchiostegal rays, meets its counterpart, fuses with it and the fused muscle is inserted on the anterior tips of the two rami of the lower jaw.

Innervation: It is innervated by the mandibular branches of the N. *maxillomandibularis* V and N. *hyomandibularis* VII and also innervated by the hyoid branch of the N. *hyomandibularis* VII.

Action: It assists in the depression of the lower jaw.

Comparison: It is somewhat similar to that of Pellona.

The Hyoid Muscles

The *adductor arcus palatini*. Fig. 30 (aap.)

Description: All the three parts of the muscle *viz.*, those inserted on the palatine, pterygoid and the hyomandibula are fused together to form one sheet of muscle.

Origin and insertion: It originates from the parasphenoid and is inserted on the palatine, pterygoid and hyomandibula.

Innervation: It is innervated by a branch of the seventh nerve.

Comparison: Unlike in Hilsa all three parts of the muscle are formed and they are fused together.

The *adductor operculi*. Similar to that of Pellona.

The *levator operculi*. Fig. 17 (lo.)

Description: It is a broad thick piece of muscle which covers the dorsal part of the operculum.

Origin and insertion : It originates in a fleshy attachment from the pterotic behind the origin of the *intermandibularis posterior* and is inserted on the dorsal half of the operculum without forming a tendon.

Innervation : It is innervated by a branch of the VIIth nerve.

Action : It assists in lifting the operculum.

Comparison : The muscle is well-developed.

The *hyohyoideus*. Fig. 19 (hs & hi.)

Similar to that of Hilsa.

The Branchial Muscles

The *levator arcuum branchialium*. Fig. 21 (labe, labi.)

They are four in number and the first one is separated into two fasciculi, the externus and the internus. All are long and narrow muscles with both the origin and insertion fleshy. The third and fourth *levatores* are fused together at their origin.

Origin and insertion : All of them originate from the parasphenoid. The first *l. externus* is inserted on the pharyngeo-branchial of the first branchial arch and the *l. internus* is inserted on the pharyngeo-branchial of the second arch. The second and the third *levatores* are inserted on the epibranchial of the fourth arch.

Innervation : These muscles are innervated by the post-trematic nerves of the IXth and Xth.

Action : They help in lifting the branchial arches.

Comparison : Only four *levatores* are developed and only the first one is separated into two fasciculi.

The *coracobranchialis*. Fig. 22 (cb.)

Description : It is very poorly developed and it is a very small muscle. Both the origin and insertion are fleshy.

Origin and insertion : It originates from the pectoral girdle and is inserted on the posterior part of the fifth branchial arch.

Innervation : It is innervated by branches of the post-trematic nerve of the Xth.

Action : As it is poorly developed, it does not help much in drawing the branchial arches back.

The *subarcuales recti*. Fig. 23 (sc.)

Description : No *subarcuales recti* are developed in any of the segments except the fourth where they occur as *subarcuales communis*.

Origin and insertion : They originate from the hypobranchials of the fifth and are inserted on the hypobranchials of the fourth arches.

Innervation : Innervated by the branches of the post-trematic nerve of the Xth.

Comparison : They assist in the depression of the ventral part of the branchial arches.

The *transversi ventrales*. Fig. 8 (tv.)

Similar to that of Hilsa.

The *Cucullaris* not developed.

The *sphincter oesophagi* is similar to that of Hilsa.

The *transverse dorsales*. Fig. 24 (td.)

Description : They are developed only in the first two branchial arches and they fuse together to form transverse sheets.

Origin and insertion : They originate from the anterior dorsal part of the *sphincter oesophagi* and are inserted on the pharyngeal branchials of the first and second arches.

Innervation : These are innervated by the post-trematic nerve of the IXth.

Action : They assist the action of the *levator arcuum branchialium*.

Comparison : Unlike in Hilsa the muscles are developed in the first and second branchial arches.

The *obliqui dorsals*. Fig. 24 (ods & odi.)

Description : The muscles are separated into *o. d. superior* and *o. d. inferior*. Both are well-developed. The origin and insertion of these muscles are fleshy.

Origin and insertion : On each side they originate from the dorso-lateral sides of the pharynx, run backwards and the *o. d. superior* is inserted on the epibranchials of the third arches and *o. d. inferior* is inserted on the epibranchials of the fourth arches.

Innervation : These are innervated by the post-trematic nerve of the Xth.

Action : To assist the action of the *levator arcuum branchialium*.

Comparison : The muscles are well-developed.

The *retractor arcuum branchialium*. Fig. 24 (rab.)

These are similar to those of Hilsa in all respects except in origin.

Here they originate from the lateral sides of the pharynx.

The Hypobranchial Muscles

The *rectus cervicis* is similar to that of Hilsa

aap	Adductor arcus palatini	MT	Metapterygoid
BR 1-5	Branchial arches	mx	maxillaris
cb	coracobranchialis	mxmnd	maxillo-mandibularis
cbe	coracobranchialis externus	O	Operculum
cbt	transverse connection of coracobranchialis	ods	obliqui dorsale superior
CH	Ceratohyal	odi	obliqui dorsale inferior
do	dialator operculi	pal q	Palato quadrate bar
hh	hyohyoideus	PO	Preoperculum
hs	hyoideus superior	PG	Pectoral girdle
hi	hyoideus inferior	PM	Premaxilla
HM	Hyomandibula	Q	Quadrate
irmn	intramandibularis	rab	retractor arcuum branchialium
inmn & ih	(ant. & post.) intermandibularis & interhyoideus	rc	rectus cervicis
lig	ligament	sc	subarcuales communis
lo	levator operculi	sp	spindle shaped region of intermandibularis
labe 1-4	levatores arcuum branchialium externus	tn	main tendon of mandibularis anterior
lap	levator arcus palatini	tn 1	tendon of the mandibularis anterior
M	Maxilla	tn 2	tendon of the mandibularis posterior
mnd	mandibularis	tv 4-5	transversi ventrales
mnd ant	mandibularis anterior	tm	trunk muscle
mnd post	mandibularis posterior		

Figs. 9 to 11 *Pellina filigera*.

Fig. 9 Side view of the head showing the cranial muscles.

Fig. 10 Ventral view of the head showing the cranial muscles.

Fig. 11 Ventral view of the branchial arches and their muscles.

Fig. 12 *Chateossus nasus*. Side view of the head showing the cranial muscles.

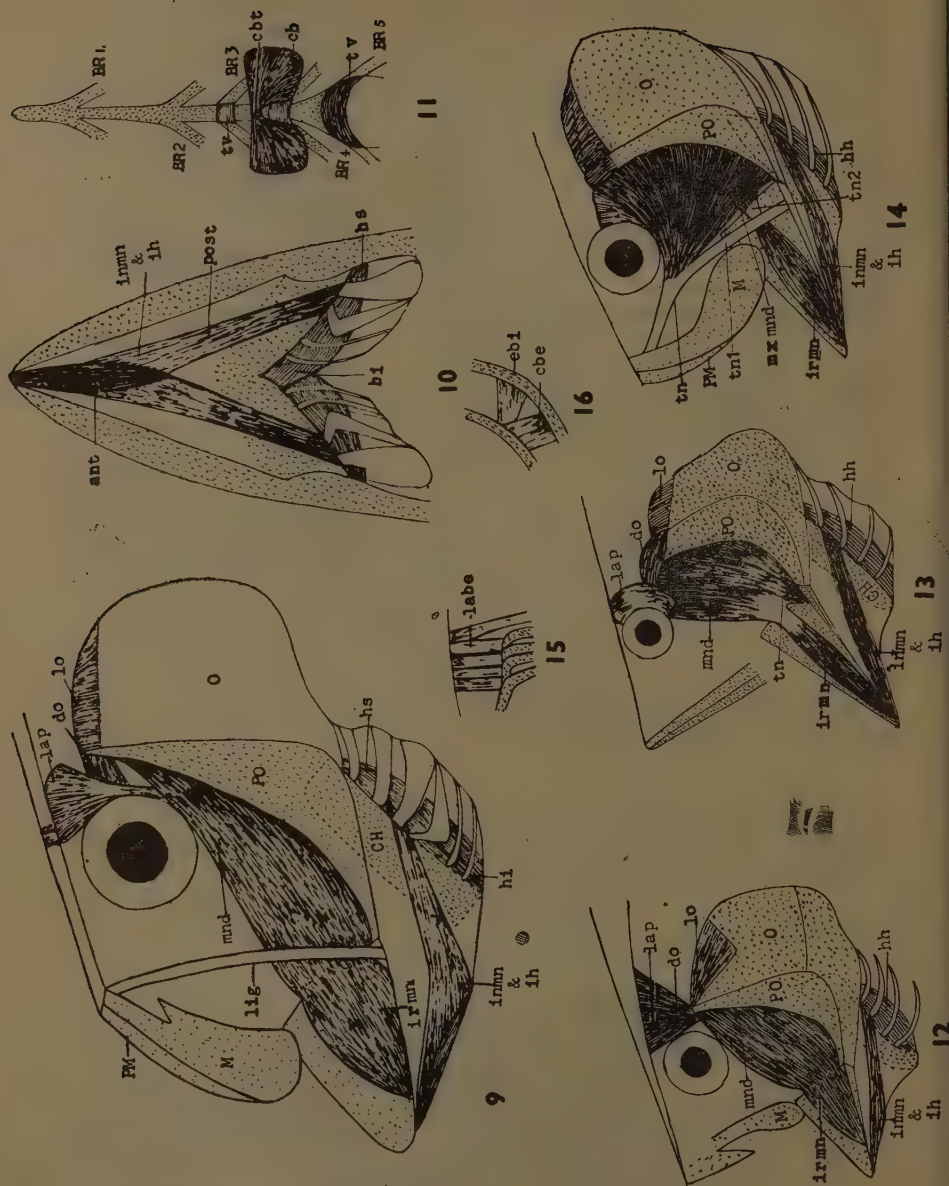
Fig. 13 *Coilia dussumieri*. Side view of the head showing the cranial muscles.

Figs. 14 to 16 *Megalops cyprinoides*.

Fig. 14 Side view of the head showing the cranial muscles.

Fig. 15 A sketch showing the origin and insertion of levatores arcuum branchialium.

Fig. 16 A sketch showing the origin and insertion of the coracobranchialis.



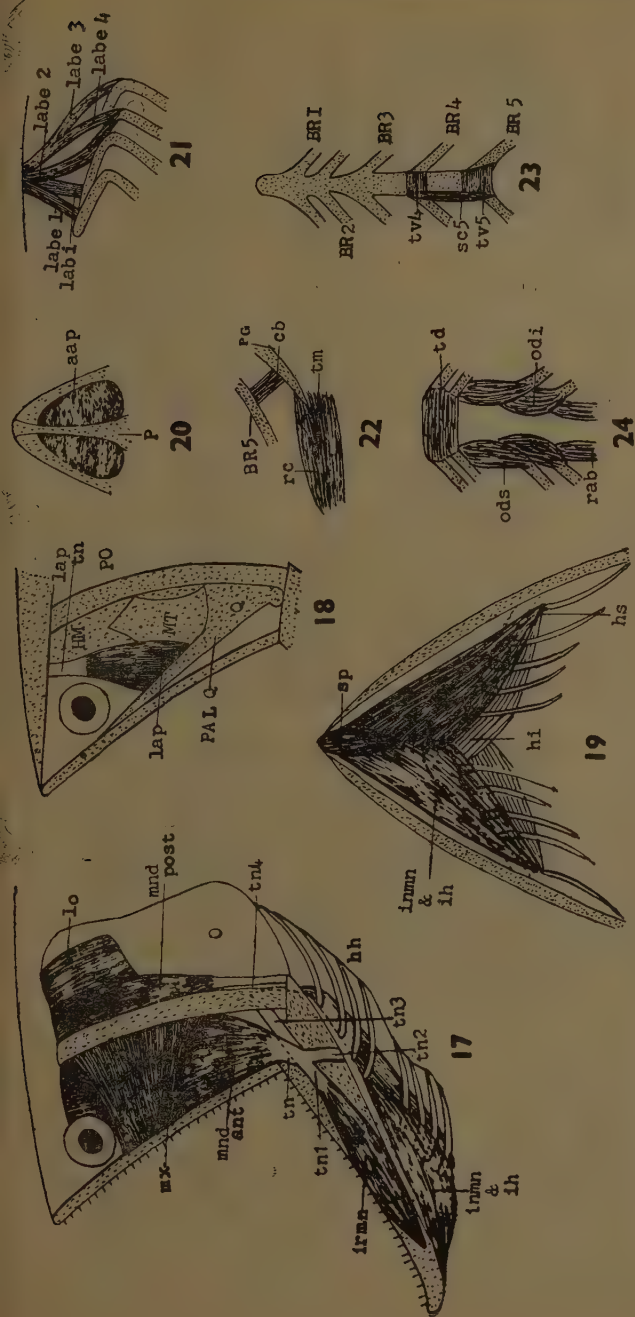
Figs. 17-24 *Harpodon nehereus*.

Fig. 17 Side view of the head showing the cranial muscles.

Fig. 18 A sketch showing the origin and insertion of levator arcus palatini.

Fig. 19 Ventral view of the head showing the cranial muscles.

Fig. 20 Roof of the mouth showing adductor arcus palatini.

Fig. 21 A sketch showing the origin and insertion of the levatores arcum branchialium.

Fig. 22 A sketch showing the origin and insertion of coracobrachialis.

Fig. 23 Ventral view of the branchial arches and their muscles.

Fig. 24 Dorsal view of the branchial arches and their muscles.

SUPER FAMILY CLUPEOIDEA

Nos.	Muscles	Family Clupeidae			Family Engraulidae
		<i>Hilsa ilisha</i>	<i>Pellona filigera</i>	<i>Chatoessus nasus</i>	<i>Cotia dussumieri</i>
1	The ligament	Connects the bases of the maxilla with the mandible	Connects the bases of the maxilla with the mandible.	Absent	Absent
2	The maxillaris	Absent	Absent	Absent	Absent
3	The mandibularis	It arises from the pro-otic, hyomandibula, symplectic, pre-operculum and the quadrate. There are two tendons arising from the anterior ventral tip.	The origin from the pro-otic and hyomandibula is lost. So it originates from the pre-operculum and symplectic. There is no tendon developed at the anterior tip of the muscle.	Similar to that of Pellona	It arises from the pro-otic, hyomandibula, symplectic, pre-operculum and quadrate. There is one tendon developed at the anterior ventral tip of the muscle.
4	The intramandibularis	It arises from one of the tendons of the <i>mandibularis</i> .	It arises from the fleshy anterior tip of the <i>mandibularis</i> .	Similar to that of Hilsa	Similar to that of Pellona.
5	The levator arcus palatini.	It arises from the frontal and descends down to the pro-otic and hyomandibula.	It arises from the frontal and hyomandibula and descends down to the pterygoid bones.	It arises from the frontal and descends down to the hyomandibula.	It arises from the frontal and descends down to the pro-otic.
6	The dialator operculi	It arises from the frontal and descends down to the pre-operculum.	It arises from the hyomandibula and pro-otic and runs backwards to the pre-operculum.	It arises from the frontal and descends to the pre-operculum.	It arises from the hyomandibula and runs backwards to the pre-operculum.

Nos.	Muscles	<i>Hilsa ilisha</i>	Family Clupeidae <i>Pellona filigera</i>	<i>Chatoessus nasus</i>	Family Engraulidae <i>Colia dussumieri</i>
7	The intermandibularis.	It fuses with the <i>interhyoideus</i> and it arises from the ceratohyal and the bases of the third and fourth brachioistegal rays.	The muscle not only fuses with the <i>interhyoideus</i> but also separates into anterior and posterior parts. Origin is the same as in <i>Hilsa</i> .	Similar to that of <i>Hilsa</i>	Similar to that of <i>Hilsa</i>
8	The adductor arcus palatini	Only two parts of the muscle are developed namely those inserted on the pterygoid and hyomandibula and they originate from the parasphenoid.	Similar to that of <i>Hilsa</i>	Similar to that of <i>Hilsa</i>	Similar to that of <i>Hilsa</i>
9	The adductor operculi	It arises from the pterotic and runs down to the operculum.	Similar to that of <i>Hilsa</i>	Similar to that of <i>Hilsa</i>	Similar to that of <i>Hilsa</i>
10	The levator operculi	It arises from the pterotic and hyomandibula.	Similar to that of <i>Hilsa</i>	Similar to that of <i>Hilsa</i>	Similar to that of <i>Hilsa</i>
11	The interhyoideus	Fused with <i>intermandibularis</i> .	Similar to that of <i>Hilsa</i>	Similar to that of <i>Hilsa</i>	Similar to that of <i>Hilsa</i>
12	The hyohyoideus	The muscle is separated into superior and inferior parts. It originates from ceratohyal and epiphyal.	Similar to that of <i>Hilsa</i>	Similar to that of <i>Hilsa</i>	Similar to that of <i>Hilsa</i>

Nos.	Muscles	Family Clupeidae		Family Engraulidae	
		<i>Hilsa ilisha</i>	<i>Pellona filigera</i>	<i>Chatoessus nasus</i>	<i>Colia dussumieri</i>
13	The levatores arcuum branchialium	It is a single sheet originating from the parasphenoid.	Similar to that of Hilsa	Similar to that of Hilsa	Similar to that of Hilsa
14	The coracobrachialis	It arises from the pectoral girdle and is inserted on the basit branchial of the fourth, the muscles from both the sides meet in a narrow piece of muscle.	Similar to that of Hilsa	Similar to that of Hilsa	Similar to that of Hilsa
15	The subarcuales recti	The muscles occur in the 2nd and 3rd branchial arches and in the fourth they occur as <i>subarcuales commutatis</i> . All are poorly developed.	The muscles are not developed in any of the branchial segments except in the fifth where they occur as <i>subarcuales commutatis</i> which are poorly developed.	Similar to that of Hilsa	Similar to that of Hilsa
16	The transversi ventrales	They are developed only in the fourth and fifth branchial segments and are poorly developed.	In the last three segments the muscles are developed. They are all narrow muscles.	Similar to that of Hilsa	Similar to that of Hilsa
17	The sphincter oesophagi	It is developed only on the ventral sides.	Similar to that of Hilsa	Similar to that of Hilsa	Similar to that of Hilsa
18	The transversi dorsales	Not developed	Not developed	Not developed	Not developed

Nos.	Muscles	Family Clupeidae			Family Engraulidae	
		<i>Hilsa ilisha</i>	<i>Pellona filigera</i>	<i>Chatoessus nasus</i>	<i>Colia dussumieri</i>	
19	The obliqui dorsales	One pair of muscles developed and each one is separated into superior and inferior parts	Similar to that of Hilsa	Similar to that of Hilsa	Similar to that of Hilsa	
20	The retractor arcuum branchialium	One pair and are poorly developed	Similar to that of Hilsa	Similar to that of Hilsa	Similar to that of Hilsa	
21	The rectus cervicis	It originates from the pectoral girdle, fuses with its fellow from the other side and also with the trunk muscle	Similar to that of Hilsa	Similar to that of Hilsa	Similar to that of Hilsa	

Acknowledgement

I take this opportunity to express my grateful thanks to Dr. C. J. George for suggesting the problem and for guidance.

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THE MYOLOGY OF THE CHELONIAN LIMB

2. The Hind Limb Musculature of *Lissemys Punctata*

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THIS is the second paper in the series. In the first paper a detailed account of the myology of the shoulder girdle and of the fore limb was given. Here the musculature of the pelvic girdle and the hind limb is dealt with.

The Muscles of the pelvic girdle and thigh :

On removing the plastron the *attrahens pelvium* and the *retrahens pelvium* (Fig. 1) are the first muscles to be encountered. They arise from the xiphiplastron and get inserted on the pubic spine and on the ventral side of the distal end of the pubis. The former acts as a medial rotator of the pelvis, while the latter acting alternately with the former acts as a lateral rotator of the pelvis. The homology of these muscles is doubtful. It is suspected that they correspond to the *rectus abdominis*. The *retrahens pelvium* is innervated by a branch from the obturator nerve, while the *attrahens pelvium* is innervated by a branch of the femoral nerve.

When these muscles are reflected, the flexor muscles of the knee are exposed. The flexor muscles of the knee have their insertion either on the tibia or the fibula. As reported by Haines (1934) and Romer (1922) these flexor muscles reach their point of insertion by three different routes. The first route is medial to the medial head of the gastrocnemius, the second one is inbetween the two heads of gastrocnemius, while the third is lateral to the lateral head of the gastrocnemius. In Varanus and Iguana (Haines, 1934) the muscles which take the first corresponding route are the *pubo-ischio-tibialis*, the *adductor tibialis*, the *flexor tibialis genicularis* and the *flexor tibialis inferior*. All these four muscles are found in *Lissemys* also with slight modification in the mode of their insertion and the point of origin, but the innervation is similar. This change in the origin and insertion of the muscles is accountable in this animal, as the skeleton and mode of locomotion in Chelonia is very highly modified. The second set of flexor muscles which reach the tibia by passing inbetween the two heads

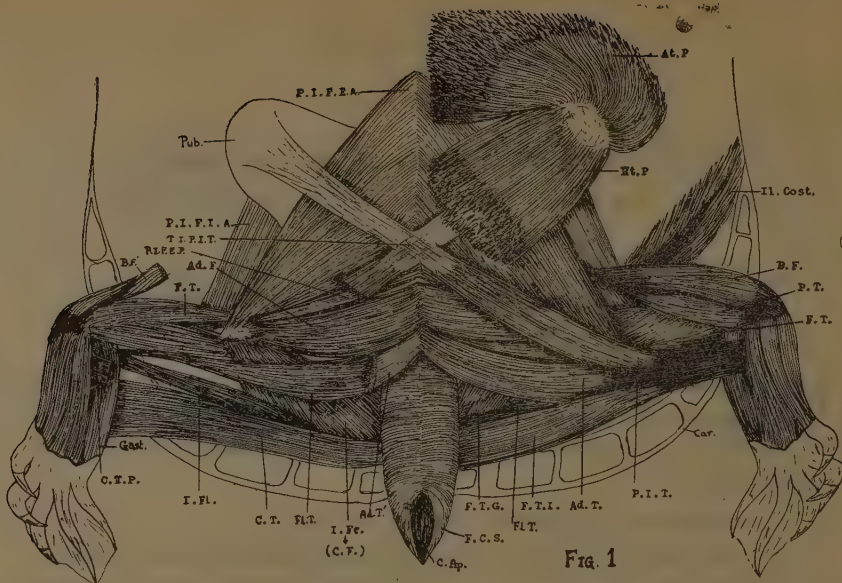


FIG. 1

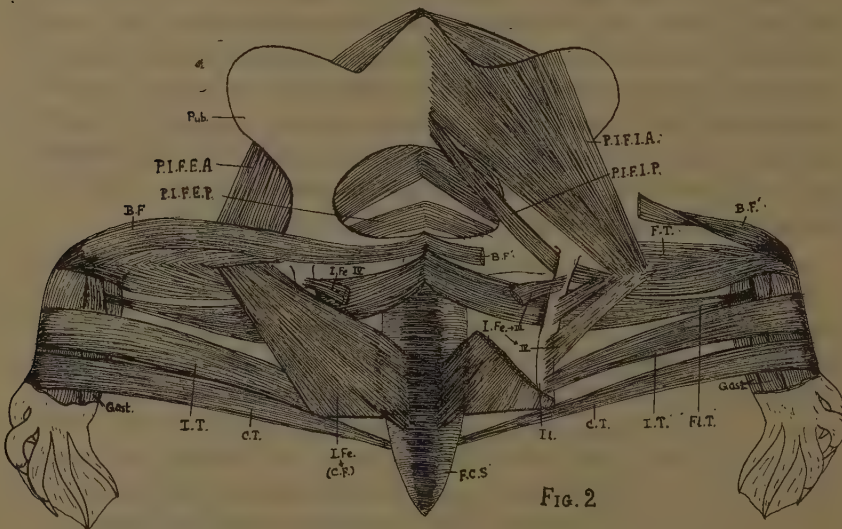


FIG. 2

Figs. 1 & 2—Muscles of the pelvic girdle and hind limb of *Lissemys*; ventral and dorsal view respectively.

Ad.T., Adductor tibialis; Ad.T', Adductor tibialis cut; At.P., Attrahens pelvium; B.F., Biceps femoris; B.F', Biceps femoris cut; Car., Carapace; C.Ap., Cloacal aperture; C.T., Caudo-tibialis; C.T.P., Cut tendon of the pubo-ischio-tibialis; F.C.S., Flexor caudae superficialis; F.T., Femuro-tibialis; F.T.I., Flexor tibialis inferior; F.T.G., Flexor tibialis genicularis; Fl.T., Flexor tibialis; Gast. Gastrocnemius; I.Fe. I—(C.F.), Ilio-femoralis (Coccygeo-femoralis); I.Fe. II, III, IV., 2nd, 3rd, & 4th part of the ilio-femoralis; I.Fi., Ilio-fibularis; Il., Ilium; Il. Cost., Ilio-costalis (not described in the text); P.I.F.E.A., Pubo-ischio-femoralis externus anterior; P.I.F.E.P., Pubo-ischio-femoralis externus posterior; P.I.F.I.A., Pubo-ischio-femoralis internus anterior; P.I.F.I.P., Pubo-ischio-femoralis internus posterior; P.I.T., Pubo-ischio-tibialis; Pub., Pubis; Rt.P., Retrahens pelvium; T.I.P.I.T., Crossing of tendons of the pubo-ischio-tibialis of either side.

of gastrocnemius in the lizards are the *flexor tibialis externus*, the *flexor tibialis anterior* and the *flexor tibialis posterior*, but in *Lissemys* there is only one single muscle mass which reaches the tibia by passing inbetween the two heads of the gastrocnemius. This muscle mass, the *flexor tibialis*, must have been compounded from the three distinct muscles in the lizards.

As in the lizards in *Lissemys* there is only one muscle, which reaches the fibula the *iliofibularis* which passes by the lateral side of the lateral head of the gastrocnemius.

The *pubo-ischio-tibialis* (Fig. 1) is the primitive muscle mass from which the mammalian gracilis is derived. The origin of this muscle is from the lateral process of the pubis by a thin, broad and membranous tendon. The tendinous strips of the muscle on either side cross and fuse with one another in the middle of the obturator foramen and then again separate and become fleshy and finally each of them unites with the *adductor tibialis*, the *flexor tibialis genicularis* and the *flexor tibialis inferior* of the same side. All these muscles together form into a single belly which later thins out into a thin membranous tendon and finally gets inserted on the lateral side of the proximal end of the tibia. The muscle acts as an adductor and medial flexor of the shank. This muscle is innervated by a branch of the obturator nerve.

The *adductor tibialis* (Fig. 1) is a massive muscle which arises along the medial line on the posterior part of the ischium. This fleshy belly joins the *pubo-ischio-tibialis* to gain a common insertion on the tibia. It is a flexor and an adductor of the shank. A branch from the tibialis nerve innervates this muscle.

The *flexor tibialis genicularis* (Fig. 1) arises from the vertebrae at the proximal end of the base of the tail. The *flexor tibialis inferior* (Fig. 1) has its origin from the vertebrae posterior to the origin of the *flexor tibialis genicularis*. These two muscle masses join to form a single massive muscle belly which finally joins with the *pubo-ischio-tibialis* and the *adductor tibialis* and have a common insertion on the lateral side of the proximal end of the tibia. All these muscles together act as the flexor and the adductor of the shank. Both muscles, the *flexor tibialis genicularis* and the *flexor tibialis inferior* are innervated by two separate branches of the tibialis nerve.

In *Iguana* and *Varanus* the second group of muscles which reach

tibia by passing inbetween the two heads of the gastrocnemius muscle consists of *flexor tibialis externus*, *flexor tibialis anterior* and the *flexor tibialis posterior* (Haines, 1934). But in *Lissemys* there is a single muscle mass instead of the three muscles mentioned above. This muscle mass, we venture to call the *flexor tibialis* (Figs. 1 & 2). It is a large massive muscle which arising from the dorsal surface of the ischial symphysis runs laterally outwards and passes inbetween the two heads of the gastrocnemius muscle and finally gains its insertion on the medial side of the proximal end of the tibia by a tough tendon, while a thin membranous tendinous slip which separates out from the main tendon gets inserted on the medial border of the distal end of the femur. This muscle acts as a flexor of the shank and is innervated by a branch from the tibialis nerve.

The last of the flexor muscles of the shank is the *ilio fibularis* (Fig. 1). It arises from the distal end of the ilium and runs laterally outwards to get inserted on the medial spinous projection of the fibula. This muscle brings about the flexion of the shank. A branch from the peroneal nerve innervates this muscle.

The *pubo-ischio-femoralis externus anterior* (Figs. 1 & 2) is a flat triangular muscle which arises from the pubic symphysis and from the ventral surface of the pubic bone. It runs posteriorly outwards and finally gets inserted on the inner trochanter of the femur by a thick tendon. This muscle brings about adduction of the thigh. This muscle is innervated by the femoral nerve; fibres from the obturator nerve also innervate the muscle.

The *pubo-ischio-femoralis externus posterior* (Figs. 1 & 2). The bellies of the muscle on the either side have their median ends joined with one another along the midventral line to form a median raphae at the level of the ischial symphysis forming the muscle mass which runs from one femur to the other. The bony origin must have been lost as the pubis having moved anteriorly and the ischium posteriorly. The insertion of the muscle is on the inner trochanter of the femur along with the *pubo-ischio-femoralis externus anterior*. The muscle acts as an adductor of the thigh. Innervation of this muscle is by a branch from the femoral nerve.

The *adductor femoris* (Fig. 1) is the primitive reptilian muscle which gave rise to the *adductor magnus* and *adductor brevis* of the mammals. Here it is found as a single muscle mass arising from the ischial symphysis

on the ventral side of the girdle and running laterally outwards it finally gets inserted on the inner trochanter along with the *pubo ischio-femoralis externus anterior* and the *pubo-ischio femoralis externus posterior*. It acts as an adductor of the thigh. A branch from the obturator nerve innervates this muscle.

The *pubotibialis* (Fig. 1) arises by a thin narrow tendinous strip from the middle of the lateral border of the pubis and runs downwards and outwards towards the leg. During its course it becomes fleshy and finally gains its insertion on the proximal end of the tibia by a flat tendon. This muscle acts as a lateral rotator and to some extent as the extensor of the thigh. The femoral nerve sends out a branch to innervate this muscle.

The *biceps femoris* (Figs. 1 & 2) is a very long flat muscle which arises from the anterior half of the ischial symphysis on its dorsal side and runs laterally outwards, covering the dorsal side of the thigh, and gets inserted on the proximal end of the tibia by a broad tendon. In mammals this muscle has two distinct heads but in this animal there is only one head at its origin. It appears that the muscle is in its primitive stage in this animal and must have differentiated into two heads at the point of origin in the mammals. The muscle in this animal acts as an extensor and to a certain extent a lateral rotator and an abductor of the shank. This muscle is also innervated by a branch from the femoral nerve.

The *ilio-femoralis* (Figs 1 & 2) consists of four bellies. One is the *coccygeo-femoralis* part which has a wide origin from the lateral side of the centre of the proximal part of the tail and the distal sacral vertebra. It is the largest belly and it runs over the ilium and then narrows down to be inserted on the outer trochanter of the femur and also on the neck of the femur. The second belly arises from the dorsal side of the middle part of the ilium it runs upwards and gets inserted on the outer trochanter of the femur along with the first belly. The third part is very small and arises just anterior to the second one from the dorsal side of the proximal end of the ilium. It runs over the neck of the femur and is inserted on the dorsal side of the inner trochanter of the femur. Lastly the fourth belly which arises from the last costal plate of the carapace, near the sacral region by the side of the vertebral column, runs laterally upwards and outwards to get inserted on the dorsal side of the outer trochanter. This group of muscles is homologous to the gluteus group of muscles and

the *tensor fascia lata* of the mammals. The action of this muscle group is medial rotation and the adduction of the thigh. Branches from the ischiadic plexus innervate this.

The *ilio-tibialis* (Fig. 2) arises in this animal from the dorsal side of the distal end of the ilium by a very narrow fleshy belly but as it runs outwards towards the tibia it widens out and later becomes membranous. It gets inserted by a thin tendon on the proximal half of the lateral border at the level of the tibia on the fascia of the shank muscles. It acts as an adductor of the shank. This muscle is innervated by branches from the peroneal nerve.

The *pubo-ischio-femoralis internus anterior* (Figs. 1 and 2) arises from the ventral surface of the pubic spine and also from most of the surface of the pubic bone on the same side. It runs obliquely backwards and outwards and finally gets inserted on the outer trochanter of the femur. There is a posterior part of this muscle which is having its origin from the ventral surface of the pubic spine and gets inserted on the outer trochanter of the femur. This part is the *pubo-ischio-femoralis internus posterior*. Both these muscles act as the abductor of the thigh. The obturator nerve innervates the two parts of this muscle.

The *caudo-tibialis* (Figs. 1 and 2) is a long muscle which arises by a very narrow origin from the lateral side of the centra of the proximal caudal vertebræ. It runs laterally outwards, widens out into a flat belly and gets inserted by a thin membranous aponeurosis on the muscles on the lateral side of the distal half of the tibia and on the skin of the foot at this level. This muscle acts as an adductor and partly as a medial rotator of the shank and is innervated by a branch from the ventral ischiadic nerve.

The *femuro-tibialis* (Figs. 1 and 2) is a massive muscle lying on the dorsal side of the thigh. It arises from the anterior surface of the ligament which connects the head of the femur to the acetabulum and also from the base of the greater trochanter and the medial and lateral surfaces of the femur anteriorly. The fibres from the medial and the lateral surfaces decussate with each other, giving a pectinate appearance to the muscle. The muscle then becomes tendinous at the level of the distal end of the femur and gets inserted on the proximal end of the tibia. It is an extensor of the shank. This muscle receives a branch from the femoral nerve.

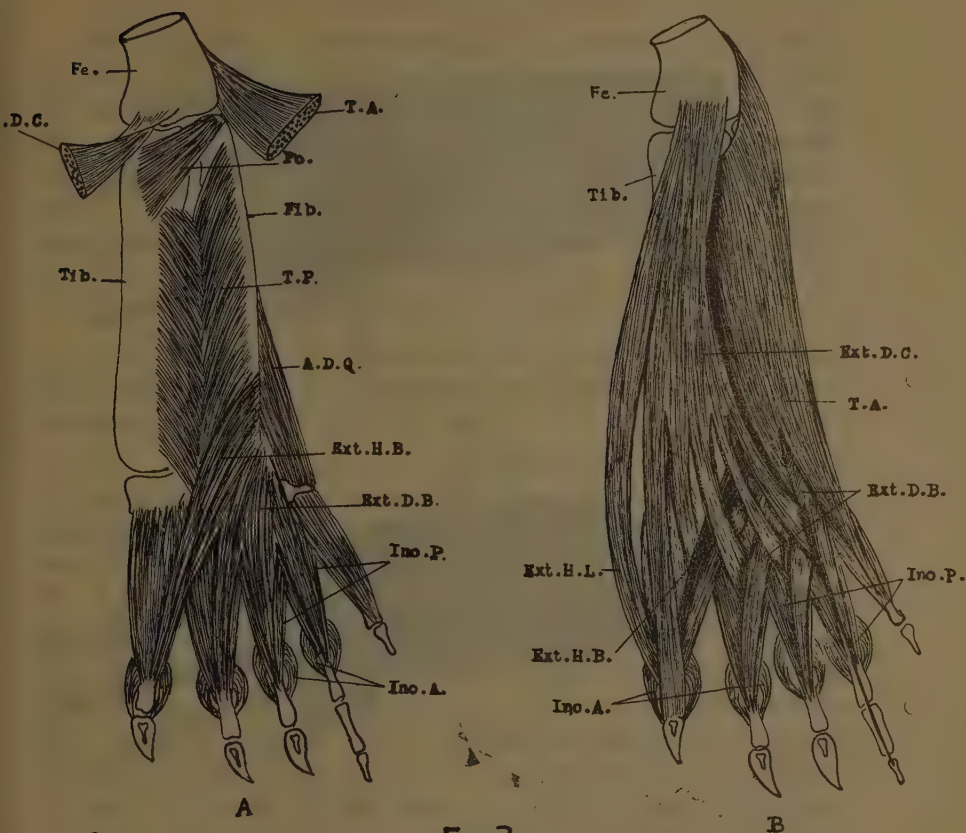


FIG. 3

Figs. 3 & 4—Muscles of the shank and foot in *Lissemys*.

H., Abductor hallucis; A.D.Q., Adductor digiti quinti; Ext.D.B., Extensor digitorum brevis; Ext.D.C., Extensor digitorum communis; Ext.H.B., Extensor hallucis brevis; Ext.H.L., Extensor hallucis longus; Fib., Fibula; F.D.B., Flexor digitorum brevis; F.D.L., Flexor digitorum longus; F.D.Q., Flexor digiti quinti; Fe., Femur; Gast., Gastrocnemius; Ino.A., Interossei anterior; Ino.P., Interossei posterior; Pero., Peroneus; Plant., Plantaris; Po., Popliteus; Sol., Soleus; T.A., Tibialis anticus; Tib., Tibia; T.P., Tibialis posticus.

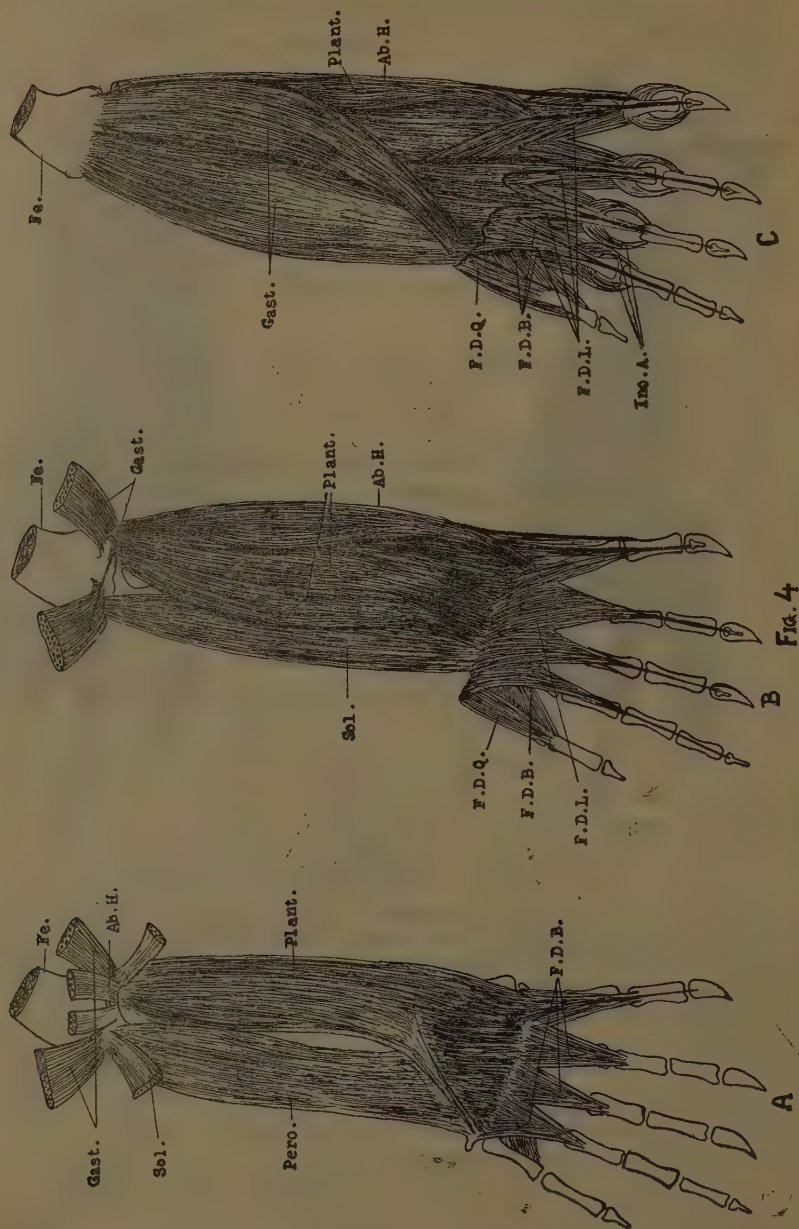


Fig. 4

The muscles of the leg and foot

The *gastrocnemius*—The muscle of the leg and foot : (Figs. 1, 2 & 4) which is a powerful calf muscle arises by two heads. One arises from the dorso-lateral condyle and the other from the medial condyle of the femur. The former consists of a larger belly than the latter. The two heads remain separate and their fibres get inserted on a broad aponeurosis which is developed on the anterior surface. The aponeurosis of both the heads unite together and finally gets inserted on the lateral and dorsal side of the intermedium. It is a powerful flexor of the foot. Branches from the tibialis nerve innervate the two bellies of the muscle.

The layer next to the *gastrocnemius* consists of the following muscles ; the *plantaris*, *soleus* and *abductor hallucis*. All these muscles receive their respective branches from the tibialis nerve.

The *soleus* (Fig. 4, a & b) is a well-developed muscle which arises from the lateral condyle of the femur and joins with the tendinous sheath of the plantar aponeurosis, which is partially inserted on the tarsals. This muscle is also a powerful flexor of the foot.

The *plantaris* (Fig. 4 a, b & c) which in the mammals is not well-developed but is found as a very thin strip, consists of two well-developed bellies in *Lissemys*. Both the bellies have a common origin from the posterior side of the proximal end and also the entire lateral surface of the tibia. They run towards the foot and unite to form a single tendinous membrane which on reaching the foot splits up into two parts—a thin and a broad one. The thin part gets inserted on the meta tarsal of the second digit, while the other forms a tendinous sheath over the other muscles in the foot, which is known as the plantar aponeurosis. It is a flexor of the foot.

The *abductor hallucis* (Fig. 4 a, b & c) is a very powerful muscle arising mainly from the medial side of the proximal end of the tibia. A few fibres arise, however, from the ligament which joins the tibia and gains its insertion on the first phalanx of the first digit by a tendon. A long thin strip from this tendon also runs forward and gets inserted on the base of the terminal phalanx of the same digit. This muscle acts as a powerful abductor of the first digit.

The *peroneus* (Fig. 4 a) consists of a single muscle mass which arises from the proximal end and also the entire lateral border of the fibula. It

is inserted by a thin tendon on the fibulare. It is also one of the flexors of the foot. A branch from the peroneal nerve innervates this muscle.

The *flexor digitorum longus* (Fig. 4 b & c) arises from the plantar aponeurosis. It spreads over the metatarsals and gives out tendinous strips one to each digit except the last one. These tendinous strips are inserted on the proximal end of the last phalanx of each of the first four digits. The muscle acts as a powerful flexor of the first four digits. The tibialis nerve innervates this muscle.

The *flexor digitorum brevis* (Fig. 4 a, b & c) arises from the tarsals and then splits up into four bellies and each one of them runs towards the digits where they get inserted on the distal end of the respective metatarsals. The last digit does not receive any part of this muscle. This muscle acts as a flexor of the digits. A branch from the tibialis nerve innervates this muscle.

The *flexor digiti quinti* (Fig. 4 b & c) arises from the fibulare and gains a fleshy insertion on the proximal end of the first phalanx of the last digit. It acts as a flexor of the last digit and is innervated by the branch from the tibialis nerve.

The muscle on the dorsal side of the leg comprise the *extensor digitorum communis*, the *extensor hallucis longus*, the *abductor digiti quinti*, the *tibialis anticus*, the *extensor hallucis brevis*, the *extensor digitorum brevis*, and the *interossei* groups of muscles. Branches from the peroneal nerve innervate all these muscles.

The *extensor digitorum communis* (Fig. 3, b) is a very prominent and well-developed muscle which arises by a narrow tendinous strip from the distal end of the femur. The tendinous part becomes muscular and flattens out and running downwards divides into four small bellies. One has its insertion on the dorsal side of the proximal end of the terminal phalanx of the first digit. The second and the third are inserted on the lateral side of the second phalanx of the second and the third digits respectively. The last one which is inserted on the entire lateral side of first phalanx of the fourth digit has also a thin tendinous part which runs on the dorsal side of the fourth digit and gets inserted on the third phalanx of the same digit. Owing to this type of insertion the *extensor digitorum communis* is more of an abductor of the hand in action than an extensor. This dual action is of great value in paddling through water.

The *tibialis anticus* (Fig. 3, b) arises from the anterior side of the proximal end of the tibia, the upper end of the fibula and from the outer side of the distal end of the femur. It runs obliquely downwards and divides into two parts. One gets inserted on the lateral side of the second phalanx of the fourth digit by a tendon, while the other also gets inserted by a tendon on the dorsal side of the second phalanx of the fifth digit. The muscle acts as an extensor and to a certain extent as an abductor of the fourth and fifth digits.

The *extensor hallucis longus* (Fig. 3, b) has a common origin with the *extensor digitorum communis*, but gets separated from it and is inserted on the ventral and the lateral sides of the second phalanx of the first digit. This muscle is situated on the extreme outer side of the *extensor digitorum communis* and acts as the extensor of the first digit.

The *extensor hallucis brevis* (Fig. 3, a) is a small thin muscular strip arising from the lateral side of the distal end of the fibula. It runs obliquely downwards and gets inserted on the inner side of the second phalanx of the first digit. The muscle besides acting as an extensor acts also as an adductor of the first digit.

The *extensor digitorum brevis* (Fig. 3 a & b) is a small muscle arising mainly from the metatarsals at the fibular end, while some fibres arise from the extreme distal end of the fibula. After its origin the muscle divides into four small strips each of which runs obliquely downwards and gets inserted on the proximal end of the third phalanx of each of the first four digits respectively. The muscle acts as an extensor of the digits.

The *tibialis posticus* (Fig. 3 a) which is the deepest extensor muscle on the shank is a thin flat muscle which has its origin by two heads. One head arises from the posterior surface of the tibia and the other from the adjacent surface of the fibula. The fibres of both decussate and then it gets inserted by a common tendon on the intermedium. It acts as the flexor of the foot. A branch from the tibial nerve innervates this muscle.

The *popliteus* (Fig. 3 a) is a small triangular muscle arising from the lateral side of the proximal end of the tibia by a very narrow strip. It runs transversely towards the fibula and gains its insertion on the median border of the proximal end of the fibula. This muscle is innervated by the tibial nerve.

The *interossei posterior* (Fig. 3 a, b) arises from tarsals and also from the proximal end of the metatarsal corresponding to each digit on the dorsal side. Those of the first two digits get inserted on the terminal phalanx of the respective digit by thin tendons. Those of the third and fourth digits get inserted by tendinous strips on the third phalanx of the respective digits. The fifth gets inserted on the proximal end of the first phalanx of the same digit.

The *ventral interossei* arises from the tarsals and the medial side of the proximal end of the first phalanx of the second, third, fourth and fifth digits. The insertion of these muscles is on the lateral side of the second phalanx of the first, second, third and fourth digits. These muscles bring about the lateral bending of the digits.

There is one more set of the *interossei* muscles the *interossei anterior* (Fig. 3 a, b) which is present on either side of the second phalanx of the first, second, third and fourth digits. Each set consists of two muscles, an abductor and an adductor which arise respectively from the medial and the lateral sides of the proximal end of the second phalanx of the first four digits. They gain their insertion on the same side on the proximal end of the third phalanx of the respective digits.

Summary

1. The muscles of the pelvic girdle and the hind limb of the pond turtle, *Lissemys punctata* is described.
2. The general plan of the musculature is typically reptilian.
3. The flexor muscles of the knee show certain interesting features:—
 - a. The *pubo-ischio-tibialis*, *adductor tibialis*, *flexor tibialis genicularis* and *flexor tibialis inferior* have different points of origin but unlike in the lizards they unite to form a single muscle mass.
 - b. The *flexor tibialis* muscle of this animal represents the *flexor tibialis externus*, *flexor tibialis anterior* and the *flexor tibialis posterior* of the lizard. These muscles in the turtle seem to be compounded from the three muscles met with in the lizards.
4. The *pubo-ischio femoralis externus posterior* is a peculiar muscle in this animal since its origin is not from a bony part but the bellies of either side have their median ends joined with one an-

other forming a median raphae which gives an appearance to these muscles as if a single muscle belly running from one femur to the other. This might have been due to the shifting of the pubis anteriorly and the ischium posteriorly leaving a wide gap inbetween.

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AMINO ACIDS IN THE DEVELOPMENT OF *PILA VIRENS* (LAMARCK)

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IN recent years embryological investigations have been laying increasing emphasis on proteins and amino acids during development. The availability of microanalytical methods like chromatography has helped the development of this new line of study in embryology.

With the aid of chromatographic analysis, the amino acids in the development of some of the vertebrate and invertebrate embryos have been investigated recently by several authors.

Holtfreter, Kozalka and Miller (1950) studied the amino acids in the eggs and embryos of several species and came to the conclusion that no new amino acids appeared or disappeared in the course of embryonic development. The free amino acids in amphibian development have been studied by Li and Roberts (1949), Eakin *et. al.* (1950), Kutsky *et. al.* (1953), Chen and Rickenbacker (1954) and more recently Deuchar (1955-56) in the *Xenopus*, and by Chen (1956) in Urodele development. Gustafson and Hjelte (1950) and Kavanau (1953-54) studied the amino acid metabolism in sea urchin embryos. Drilhon and Busnel (1950) investigated the free amino acids in the eggs of *Bombyx mori* and Shaw (1955) in grasshopper egg.

These different investigations have not shown much unanimity of results. We mentioned already the conclusion of Holtfreter, Kozalka and Miller (1950). Kutsky, Eakin, Berg and Kavanau found that there was no overall pattern of change of amino acids. On the other hand Berg (1950) found that in the development of *Strongylocentrotus purpuratus*, alanine, glutamine and lysine progressively declined.

Gustafson and Hjelte (1950, 51) found in *Paracentrotus lividus* only slight changes during cleavage, but important synthetic changes initiated at gastrulation as indicated by a drop in free amino acids and rise in peptide amino acids,

Kavanau (1953) found that protein-bound amino acids decline continuously until hatching when there is a sharp drop. The same author later studied *Paracentrotus lividus* and found cyclic changes in protein-bound amino acids suggestive of alternative periods of yolk degeneration and tissue protein synthesis. Deuchar (1956) who investigated *Xenopus* with a view to obtaining information about embryonic amino acids in relation to differentiation of embryonic proteins could not obtain incontrovertible evidence that amino acids become distributed in the embryo in the regions where they are required most for synthesis of specific proteins.

The problem in *Pila* is somewhat different. Here there is a reserve nutritive fluid surrounding the embryo and containing free as well as protein bound amino acids (Ramamoorthi, 1955). During development these substances are taken up by the embryo and the problem, therefore, is the utilisation of these reserve proteins and amino acids. The present study relates to the free and protein-bound amino acids in the several stages of development of the apple-snail.

Methods

Two hundred embryos were taken for each determination. The different stages were analysed as shown in Table 1.

For the study of the free and protein-bound amino acids circular filter paper chromatographic technique as described by Giri *et. al.* (1952) with Whatman paper Nos. 1 and 20 was adopted. n-Butanolacetic-water mixture (4:1:5) was used as the solvent and 0.1% ninhydrin as colour-developing reagent.

For the identification of the different amino acids mixed chromatograms as described by Giri and Rao (1952) were run, by spotting both the known and the unknown in the same chromatogram. Multiple development technique as described by the same authors was adopted to identify amino acids which failed to separate as distinct bands. Spot tests were also carried out for some of the overlapping amino acids.

For the separation of free amino acids, the embryos were ground with sufficient quantity of absolute alcohol to precipitate the protein. Then after centrifugation the supernatant liquid was treated with thrice the quantity of chloroform, centrifuged again, and the upper layer alone was used for chromatographic analysis.

For the preparation of the hydrolysates the procedure described by Giri *et. al.* (1952) was adopted.

Results

At the commencement of development in the freshly laid egg, the free amino acids are present in low concentration, appearing as faint bands and include alanine, threonine-glutamic acid, and glycine-serine-aspartic acid.

In the two-celled stage there appears in addition hydroxyproline which is confined to this stage and absent from all succeeding stages of development. The rest of the cleavage stages as well as the blastula and trochophore stages do not show any additional free amino acids.

In the early veliger on the third day of development when the foot is beginning to appear as a slight conical protruberance and the shell gland is still invaginated, three more amino acids leucine, isoleucine and phenylalanine appear and are recognisable in the chromatogram as feeble bands. On the fourth day the foot rudiment appears as a distinct conical projection, and the shell-gland is everted and covered with a thin cuticular shell and the cephalic vesicle of the embryo increases in size. Two more free amino acids, valine and methionine, appear during this stage. On the seventh day still three more amino acids, tyrosine, arginine and asparagine, are added to the free amino acid pool. During this day among other changes the mantle edge becomes pigmented, tentacle and eyes are well-developed and the foot has almost a definitive shape and provided with an operculum. These amino acids which appear on this day are in low concentration but the other amino acids, present since the commencement of development, namely alanine, threonine, glutamic acid, glycine, serine and aspartic acid now increase in concentration and appear as intense bands in the chromatogram.

In the subsequent days of development from the seventh to the time of hatching, important morphological changes take place, leading to definitive organisation. During this period proline is added to the pool of free amino acids which now number fifteen appearing in ten bands in the circular chromatogram.

Protein-bound amino acids

At the commencement of development only two amino acids, glutamic acid and glycine are present in the fertilised ovum. In the four-celled

stage hydroxyproline, arginine and histidine are present in addition. In the third day embryo—when several organ rudiments appear—eleven more amino acids appear *viz.* leucine, isoleucine, valine, methionine, proline, alanine, threonine, serine, aspartic acid, lysine and ornithine. During the fourth day tyrosine appears. On the sixth, phenylalanine, and on the eighth, asparagine appear. Cystine appears in the embryo before hatching. By the time all the definitive organs begin to function, the increase in protein-bound amino acids is finalised and ceases.

Discussion

The egg of *Pila* is provided with reserve nutritive material in the form of the so-called albuminous fluid. In a previous study (Rama-moorthi, 1955) it was shown that all the free amino acids as well as protein-bound amino acids of the nutritive fluid either become very low in concentration or completely disappear by the trochophore stage. They are evidently utilised in the developing embryo either directly or indirectly. It is to be expected that the disappearance of amino acids from the nutritive material should be related to the appearance of new amino acids in the successive stages of development of the embryo.

The observations recorded in the previous section show that the glucogenic amino acids—alanine, threonine, glutamic acid, glycine, serine and aspartic acid—are present in the free amino acid pool in all the stages beginning from the fertilized egg. But only two protein-bound amino acids, glutamic acid and glycine are present in the fertilized egg. Practically no changes are observed in the free as well as protein-bound amino acids during cleavage, blastulation and even gastrulation. This would indicate there is no protein synthesis during these stages, which are concerned only in the distribution among the different blastomeres the materials already present in the egg. However, hydroxyproline as a free amino acid appears in the two-celled stage, and as a protein-bound amino acid in the four-celled stage. This is difficult to interpret. It may be that it is synthesised from part of proline which is present in the nutritive material till the 8-celled stage. According to Thorpe (1953) experiments with proline labelled with D and N¹⁵ suggest that it can be converted into glutamic acid, ornithine and hydroxyproline.

Leucine and isoleucine, which are present in the nutritive fluid, first appear in the embryo as free amino acids and also as protein-bound amino

TABLE I
Free and protein-bound amino acids in different stages of development of *Pila Virens*

[illegible]

Amino acids	Fertilised egg												P	F
	2-celled	4-celled	cleavage	Blastula	Gastrula	Trocho-phore	3-days	4-days	6-days	7-days	8-days	Before hatching		
Glycine (Gly), Serine and Aspartic acid	Gly	Gly	Gly	Gly	Gly	Gly	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Asparagine														
Arginine														
Histidine														
Lysine and Ornithine														
Cystine														

P. Protein-bound amino acid
F. Free amino acid

acids on the third day when organ rudiments arise. Phenylalanine, which also is present in the reserve material outside the embryo appears in the embryo as a free amino acid on the third day and as a protein-bound on the sixth. Valine and methionine also present at first outside the embryo appear inside it on the third day in the protein-bound form and on the fourth day in free condition. Tyrosine also can be traced to the extra embryonic source and appears in the embryo on the fourth day as a protein-bound acid, and on the seventh day as a component of the free amino acid pool. On the seventh day, as we said above, the appearance of tentacles, eyes and pigmentation are characteristic and it is probable that there is intensive metabolism related to it. Asparagine appears on the seventh day as a free amino acid and about the eighth as protein-bound. Arginine appears on the seventh day as a free amino acid, and during cleavage as a protein-bound amino acid. Cystine is present in the 'albuminous' fluid till 32-celled stage but not in the embryo either in the free or protein-bound condition. However, it appears in the embryo just before hatching. The sulphur-containing amino acids are represented in the embryo only by methionine which appears early (third and fourth days) in the embryo in the protein-bound and free conditions. It might be that all the cystine of the albuminous fluid is transformed into methionine, but this would require vitamin B₁₂ and folic acid for the synthesis of methionine from homocysteine.

These findings relating to the changes in the amino acids during the development of *Pila* tend to show there is some synthesis of proteins during development. There is some evidence of not only the utilisation of the free amino acids, but to some extent breakdown and synthesis of new proteins from the proteins and amino acids derived from the reserve nutritive material.

Acknowledgements

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HISTOCHEMISTRY OF MUSCLE LIPASE

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[1 Plate]

THE occurrence of lipase in the skeletal and cardiac muscles of vertebrates was reported in our earlier papers (George and Scaria, 1956 & '57). The present study was undertaken to demonstrate the presence of the enzyme in the muscle by histochemical methods.

A variety of techniques are available for the histochemical demonstration of esterases in tissues. The differences in these methods are largely due to substrates. The choice of substrates is important, because of the specificity of substrates exhibited by many esterases which enables one to distinguish one esterase from another. Since there is a certain degree of overlapping of substrate specificity between the nonspecific esterases and lipase the choice of a suitable water soluble substrate for lipase presented great difficulties in the past. In such cases advantage was taken of the observation that certain chemicals inhibit one or other of the enzymes while the remaining is either unaffected or activated. A thorough discussion on the merits and demerits of the various methods is available in recent literature on the subject (Pearse, 1954; Gomori, 1953; Zacks and Welsh, 1953). Gomori (1945) devised a method for lipase in which he used the Tweens as substrate. Later he pointed out that the saturated Tweens are general substrates for lipase-esterase and recommended for true lipase Tween 80 which contain unsaturated fatty acid. This Tween method for lipase which has undergone several modifications at the hands of Gomori himself and many others form the basis for our present study. The principle of this method is that the fatty acids liberated as a result of the enzymic activity of lipase is converted to insoluble calcium soap by the calcium ions present in the incubation medium. The calcium soap thus formed is subsequently converted to lead soap and ultimately to lead sulphide by treatment with lead nitrate and ammonium sulphide respectively. On microscopic examination of

the sections the lead sulphide is seen as brownish black deposits at the sites of enzymic activity.

Material and Methods

Transverse sections of the *pectoralis major* muscle of the kite (*Milvus migrans*), the green parakeet (*Psittacula kramari*), the brown dove (*Streptopelia senegalensis*), and the heart muscle of the blue rock pigeon (*Columba livia*) were studied. The pancreas of the pigeon was used for comparison and confirmation of the results.

The incubation medium contained 2 ml. 10% CaCl_2 , 2 ml. 5% Tween 80 (Atlas), 5 ml. bicarbonate buffer (Sneath, 1951) of pH 8.4 and 40 ml. distilled water. The mixture was incubated overnight at 40°C to precipitate the free fatty acids, filtered through No. 1 Whatman filter paper to remove the precipitate and a crystal of thymol was added as preservative.

Preparation of the Sections

A critical review of the various methods which have been in use for enzyme studies is presented by Pearse (1954). Gomori originally used paraffin sections of acetone fixed tissues. Later literature shows the use of frozen sections of formalin fixed tissues by various authors (Mark, 1950; Seligman *et al*, 1951). In our hands acetone fixing and subsequent paraffin embedding yielded negative results which we thought was due either to the denaturation of the enzyme by acetone or its inactivation by heat at 58°C which was the melting point of the paraffin wax used. In order to verify this, parallel experiments were run with sections of the pancreas. The results were negative in either case. It was also observed that even with the use of paraffin wax of low melting point the results were found to be negative and was therefore concluded that the inactivation of lipase was largely due to its denaturation by acetone. This is in agreement with Mark (1950) who observed that acetone fixation destroys a large amount of lipase. This view is further substantiated by our observation (unpublished) that the acetone powder of the pigeon breast muscle is almost completely inactive.

Fixation of tissues in buffered or neutral formalin in cold has been in use for histochemical study of enzymes. Seligman *et al* found that under the above conditions an appreciable amount of enzyme is left undestroyed. In pancreas at least 75% of lipase was retained. Following the recommendation by Pearse (1954) "As a starting point for further research on

the Tween methods a standard 16-hour fixation in 6 per cent neutral formalin at 4° should be used" we used 6% cold neutral formalin as fixative. But instead of cutting frozen sections of tissues after formalin fixation what we did was to cut fresh frozen sections of tissues and to fix them in formalin after mounting on slides. This procedure is more advantageous in that the time taken to remove the formalin after fixation is considerably reduced.

In histochemistry the preparation of the sections is of utmost importance for obtaining good results. We could do no better than quote Pearse (1954), "The effect of fixation on enzymes continues to be of increasing importance as histochemical techniques for demonstrating them increases in numbers. Theoretically the best way of showing enzyme activity by histochemical means would appear to be by the use of fresh frozen sections of tissue blocks. There are formidable objections to both in practice, however, especially to the former. Even with the cold knife method it is technically impossible to obtain good fresh frozen section of the majority of tissues, and even if these are obtained or if thin tissue blocks are substituted there remains a further objection. Particularly if incubation with the substrate is prolonged I have observed that loss of enzyme and of other protein and non-protein materials into the incubating medium is much greater than in fixed material. This leads not only to false localization of enzyme (due to diffusion in the section) but also to widespread deposition on the section of the products of enzymic activity in the medium and to general filthiness of the sections due to partial dissolution of its components." Our experience with the freezing microtome was that it is very difficult to mount good thin sections on the slide without transferring them from the knife into water. This procedure is not desirable because much of the soluble enzymes are likely to be lost in water during this process. But a very simple and quick method which does not need a cold room or elaborate instruments, as described below, was found to overcome this difficulty and yield highly satisfactory results. Tissues from animals are cut out immediately after killing and placed over ice in the deep freeze of a refrigerator. The tissues freeze hard in less than half an hour. Without removing them from the deep freeze they are trimmed and thin sections cut with the aid of a cold sharp razor blade. If the blade is completely dry, the sections do not stick to it. The sections thus cut are transferred to a slide over albumin, if desired, by means of a cold needle or with the corner of the blade itself, where due to the melting of the ice the sections get spread out. One can

be certain that by this method no enzyme will be lost. Perhaps the only serious drawback in this method is that it will be difficult to determine exactly the thickness of the sections and to get sections of uniform thickness throughout. But this method could be employed admirably for qualitative histochemical work on enzymes. The sections thus prepared may be fixed in cold formalin. However this precaution does not guarantee exact localization of the enzyme since the possibility of diffusion of the products of enzymic activity still remains. It was observed that in the case of the muscle lipase, satisfactory results could be obtained only with sections 40-80 microns thick. Opaqueness of the sections due to ammonium sulphide can be removed by keeping the sections for a few seconds in a Coplin jar of 70% alcohol mixed with a few drops of hydrogen peroxide. Incidentally it may be mentioned that the above method of cutting sections can conveniently be used for manometric studies of tissue metabolism and also for the analysis of tissue components by paper chromatography.

Procedure

Sections of tissues from animals which were bled to death were cut as described above and mounted on albuminised slides and allowed to dry at room temperature till adhesion was complete. They were then fixed for 16 hours in cold formalin (4°C) neutralised with phosphate, washed in running water filtered through cotton for 1 hour, rinsed with distilled water and incubated for 8-12 hours. The rest of the procedure was essentially the same as described by Pearse (1954). The sections were not counterstained.

Control

The enzyme was inactivated by keeping the slides in boiling water for about 10 minutes.

Results and Discussion

Except in the case of controls all the preparations showed brownish black precipitates indicating lipase activity (Plate 1). Naturally the greatest activity is manifested in the sections of pancreas (fig. 1) by a thick deposition of the precipitate. The heart muscle (fig. 2) comes next in the order of activity. This is in conformity with the results obtained in quantitative determinations (George and Scaria, 1957) which showed that the heart muscle contain a higher concentration of the lipase than the breast muscle. The distribution of lipase in the *pectoralis major* muscle of the three birds is interesting. In both kite and parakeet the *pectoralis major* consists of fibres which are more or less uniform with regard to their fat content

(George and Naik, unpublished) and it is seen that the lipase also enjoys a uniform distribution throughout the fasciculus (Figs. 3 & 4). On the other hand in the dove the *pectoralis major* consists of two distinct types of fibres, a broad white variety with clear cytoplasm and a narrow red variety with lot of lipid inclusions a condition very similar to that in the pigeon and bat. In these animals it has been observed that the lipase activity of the *pectoralis major* muscle is confined only to the narrow fibres (George and Scaria, 1958; George, Sushila and Scaria, 1958). In the case of the dove too as can be seen from the photograph (fig. 5) only the narrow fibres contain the precipitate in abundance. The few grains of the precipitate found scattered here and there in the broad fibres, without doubt, are due to the diffusion of the products of hydrolysis from the narrow fibres, as could be seen from an examination of a number of sections. It may be mentioned here that a histochemical study of certain of the enzymes of Krebs cycle and glycolysis in pigeon breast muscle showed that all the enzymes are confined to the narrow fibres (George, Nair and Scaria, 1958 and George and Scaria, unpublished). A correct interpretation of the functional significance of the two types of fibres in the breast muscle of these animals should however await further investigation and such are in progress in our laboratories. Another important fact that has emerged from our present study is regarding the nature of the fat splitting enzyme in the muscle. Mention was made in the beginning about the confusion in nomenclature of the fat splitting enzymes. Since the substrate we used in our present study is one specific for true lipase and the results obtained are comparable to that of pancreas, it confirms our view, based on the study of substrate specificity by other methods and the effect of inhibitors (unpublished) that the muscle enzyme is a true lipase and not an esterase.

Summary

1. Using Tween 80 as substrate, the presence of a true lipase in the heart muscle of the pigeon and the *pectoralis major* muscle of the dove, kite and parakeet is demonstrated.
2. In the dove, the enzyme is confined to the narrow fibres while it is uniformly distributed throughout the muscle fasciculus in the kite and parakeet.
3. The muscle lipase is destroyed by acetone fixing.
4. A simple method for cutting fresh frozen sections of tissues is described.

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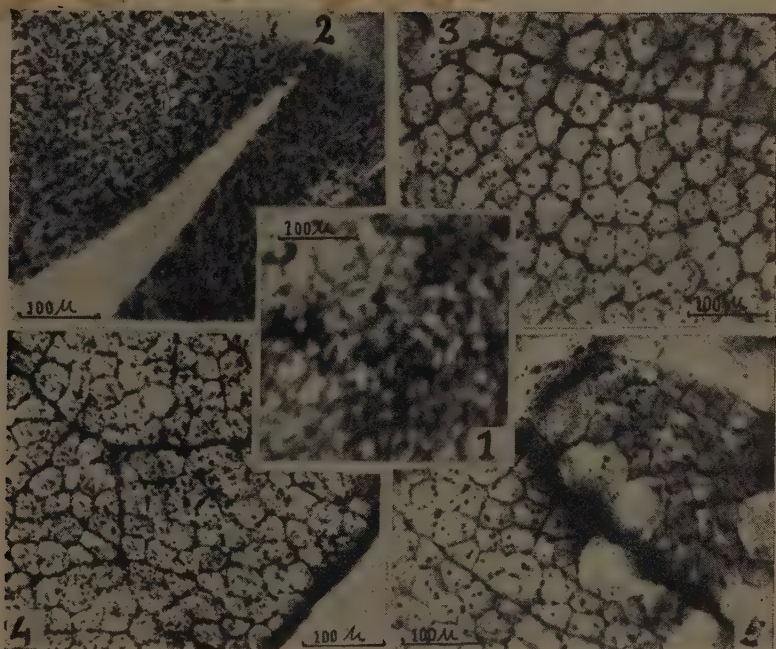


Plate I

Disposition of lipase in animal tissues. Fig. 1. T. S. pigeon pancreas, Fig. 2. L. S. pigeon heart muscle. Figs. 3, 4 and 5. T. S. *pectoralis major* muscle of the kite, parakeet and dove respectively.

CERTAIN HISTOCHEMICAL AND PHYSIOLOGICAL OBSERVATIONS ON THE ADIPOSE TISSUE OF THE PIGEON

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THE adipose tissue of mammals especially rats have been the subject of extensive studies in recent years. It has been shown that adipose tissue is a metabolically active tissue (Feller, 1954; Hook and Barron, 1941; Schoenheimer, 1946), and not an inert one as was thought before. Whereas in rats and certain other animals there are two types of adipose tissues designated brown and yellow, of which the brown is more active metabolically (Fawcett, 1952), in pigeon and other birds only the yellow type is known to be present. The adipose tissue in birds is of especial interest since migratory birds are known to store large amounts of fat prior to migration (Odum and Perkinson, 1951; McGreal and Farner, 1956). The fat that is stored is mostly spent up during the migratory flight. All the information that we have at present concerning the adipose tissue in birds, is some data on fat depletion in this tissue of migratory birds and the nature of the fat it contains (McGreal and Farner, 1956; King and Farner, 1956; McGreal, 1956; Hilditch, 1949; Hilditch *et al*, 1934; Nutter *et al*, 1943). But no serious attempt has ever been made to find out and assess the metabolic activity that takes place in this tissue. Since it has been shown that fat is the chief source of energy for long and sustained muscular activity (George and Jyoti, 1957; Weis-Fogh, 1952) it necessarily follows that the adipose tissue in such animals should play a vital role in metabolism. Since the pigeon is also known to utilize fat for muscular activity during flight (George and Jyoti, 1957), a study of the adipose tissue of this animal was undertaken to find out the role of this tissue in the energy metabolism of the animal. This paper deals with a study of the histology, vascularity and certain enzymic activities of the adipose tissue of the pigeon. The following enzymes were studied: lipase, alkaline phosphatase; succinic, malic, lactic, glycerophosphate, D-glucose, and glutamic acid dehydrogenases.

Material and Methods

The material used was the visceral adipose tissue (from the ventral side covering the coils of the intestine) of the blue rock pigeon (*Columba livia*). It was our experience that when pigeons are well fed in the laboratory for about two weeks the visceral adipose tissue grows to enormous bulk and provides excellent material for such study. This tissue was removed after decapitating the animal to ensure that much of the blood was drained off.

Normal Histology

For the study of normal histology, tissues were fixed in Zenker's fluid for 1 hour, washed in running water for the same time, dehydrated and embedded in paraffin. Sections were cut at 10μ and stained with haematoxylin and eosin. Few sections were stained with haematoxylin and Van Gieson stain for the demonstration of collagen.

Blood Supply

5 ml of India ink was injected directly into the left ventricle of a live pigeon under ether anaesthesia. The animal died after a short time. It was then fixed whole in 10% formalin for 24 hours and the adipose tissue removed. Observations on the extent of blood supply were made from frozen sections of this tissue.

Enzymes

All the enzymes were studied histochemically except lipase which was studied histochemically as well as quantitatively.

Quantitative Determination of Lipase

Lipase activity of the adipose tissue was manometrically determined quantitatively according to the method followed by Martin and Peers (1953) in a bicarbonate, CO_2 buffer system of pH 7.4 at 37°C using the Warburg manometric apparatus (Umbreit *et al*, 1951). The reaction flask contained 1.5 ml 0.025 M bicarbonate solution 0.5 ml distilled water and 0.5 ml enzyme solution in the main chamber and 0.5 ml 4% (v/v) tributyrin in 0.0148 M bicarbonate solution, emulsified by shaking with a drop of Tween 80, in the side arm thus making up a total volume of 3 ml. The manometer and flask were gassed with a mixture of 5% CO_2 and 95% N_2 from a cylinder. After equilibration for 10 minutes the contents of the side arm was tipped in and the readings taken at an interval of 15 minutes for 1 hour. The enzyme material used was an aqueous extract of ether

defatted adipose tissue which was prepared in the following manner. Fresh adipose tissue was minced and kept in ethyl ether at room temperature. After extraction of fat for 12 hours the ether was decanted and the tissue dried in vacuum in a dessicator at room temperature. Much of the fat was removed by this process. 50 mg of the dry tissue was then weighed and extracted in cold water for 1 hour by scrubbing with sand. It was then centrifuged at about 2500 r.p.m. for 5 minutes and the supernatant used as the enzyme solution. This enzyme solution contained approximately 4 mg of protein/ml. The lipase activity is expressed as μ moles butyric acid liberated/hour/mg protein of the enzyme solution. Protein was estimated using the micro-Kjeldahl method for total proteins (Hawk, 1954).

Histochemical Methods

Lipase : Lipase activity of the adipose tissue was studied on fresh frozen sections according to the procedure for muscle lipase described by George and Scaria (1958) with the modification that the sections were defatted in ethyl ether for 10 minutes prior to fixing. This modification was necessary because undefatted sections did not yield good results. The method employed was the Tween method of Gomori using Tween 80 as substrate. Boiled sections were used as control.

Alkaline Phosphatase : Alkaline phosphatase was studied according to the revised method of Gomori using sodium glycerophosphate as substrate. Fresh sections were cut according to the method of George and Scaria (1958), transferred to clean slides and allowed to dry at room temperature. After defatting for 10 minutes they were fixed in 10% cold formalin (4° C) for 2 hours, washed in running water for 30 minutes, rinsed with distilled water and incubated in the substrate medium (Pearse, 1954) at 40°C for 24 hours. The rest of the procedure was the same as described by Pearse. Sections which were kept in boiling water for 10 minutes prior to incubation was used as control.

Dehydrogenases : Dehydrogenase activity of the adipose tissue was studied following the method of Straus *et al* (Pearse, 1954) using 2:3:5 triphenyl tetrazolium chloride as the hydrogen acceptor. Fresh sections were cut as for alkaline phosphatase, into cold 0.1 M phosphate buffer of pH 7.2. After keeping them in the buffer for 15 minutes to ensure that all the endogenous activity is lost, they were transferred into the respective incubation mixtures and incubated for 10 minutes at 40°C. Incubation mixture in each case contained 1.5 ml of phosphate buffer of

pH 7.2, 1 ml of 0.1 M solution of the substrate, 7.5 mg of 2:3:5 triphenyl tetrazolium chloride and 0.625 mg of DPN in a total volume of 2.5 ml. DPN was omitted from the incubation mixture for succinic dehydrogenase because this enzyme does not require the coenzyme.

Results

Normal Histology

Figure 1 presents the photomicrograph of a section stained with haematoxylin and eosin showing the gross histology of the adipose tissue. The cell walls appear to be made up mainly, if not solely, of collagen. The whole tissue is enveloped in a thick layer of collagen as revealed by sections stained with haematoxylin and Van Gieson stain. In a number of sections studied there were many erythrocytes scattered throughout the cytoplasm.

Blood Supply

There is a plentiful supply of blood to this tissue as seen from the abundance of the capillaries. Figure 2 presents a photomicrograph revealing the distribution of the capillaries.

Enzymes : Quantitative

Lipase : The enzyme activity is approximately 11 μ moles of butyric acid/mg protein/hour. This is many times higher than that of fat free dry tissue of the *pectoralis major* muscle of the same animal (George and Scaria, unpublished).

Histochemical

Lipase : Figure 3 is the photomicrograph of the adipose tissue showing lipase activity. The sites of enzyme activity were seen as brownish black precipitate. The abundance of the precipitate is due to the high lipase activity as also shown by the quantitative estimation of this enzyme.

Alkaline phosphatase : Figure 4 presents the photomicrograph of a section treated to demonstrate alkaline phosphatase activity. The tissue was coloured deeply (black) showing high enzyme activity. Nuclei and capillary endothelium stands out in activity as shown by the intensity of the colour. This is in conformity with the observations of Fawcett (1952) on the adipose tissue of rats.

Dehydrogenases : All the dehydrogenases tried were found to be present except D-glucose dehydrogenases. Dehydrogenase activity was detected

by the pink colour developed in the cells. The exact localization of the enzyme activity was not possible because formazan produced by the reduction of 2:3:5 triphenyl tetrazolium chloride went in solution in the fat which is abundant in the tissue.

Discussion

That "the histological structure of adipose tissue has been studied less extensively than that of many other tissues because it was long regarded as a storage form of connective tissue with little physiological activity of its own" has been rightly stated by Fawcett (1952). In his studies on brown and yellow adipose tissues of rats he (1952) has reported the presence of a rich blood supply to the brown adipose tissue. In the pigeon where there appears to be only the yellow adipose tissue, blood supply to this tissue is very rich. This is indicative of its high metabolic activity. Feller (1954) investigating the incorporation of radioactive acetate carbon into lipids of adipose tissue and liver in rat has reported a higher activity for adipose tissue. He states, "When the values obtained for synthesis of fatty acids are reduced to cytoplasmic unit, namely fat-free tissue or total nitrogen content, adipose tissue can form fatty acids at rates equal to or greater than those rates found for liver slices." This provides further evidence for the fact that adipose tissue taken as fat-free tissue is metabolically equal to if not more, than other tissues known to be very active. Our quantitative study of lipase activity in the adipose tissue, also substantiates this view.

For the histochemical study of lipase Tween 80 was used instead of Tween 40 or Tween 60, as substrate. Fawcett (1952) has expressed doubt as to the presence of true lipase in adipose tissue from his studies on rat adipose tissue using Gomori method (Tween 40 and Tween 60 as substrates). Tween 80 is found to be attacked only by true lipase and not esterase (Gomori, 1953). Since in our study we used Tween 80 as substrate it can be said with certainty that the lipolytic enzyme of the adipose tissue is a true lipase. This does not disprove the occurrence also of other esterases. Quantitative estimation of lipase revealed a high concentration of this enzyme in adipose tissue. This high concentration of lipase is necessary for the synthesis and break down of the large amounts of fat present in this tissue. This might explain the rapid depletion of much of the fat in adipose tissue during migration. During some experiments conducted in our laboratory we have noticed the depletion of much

of the fat in the adipose tissue of the pigeon during continuous muscular exercise (George and Eapen, unpublished). Schoenheimer (1946) in his studies using isotopes as biological tracers have shown that lipids stored in adipose tissue are not static reserves but are constantly being drawn upon and restored.

The presence of the enzymes of the Krebs cycle suggests that adipose tissue takes an active part in the metabolic activities along with other known active tissues, possibly in the synthesis of fatty acids from carbohydrate which is the main constituent of the animal's diet. The occurrence of lactic dehydrogenase is significant from the fact that normally there is no reason why lactic acid should be produced in the adipose tissue. It is possible that part of the lactic acid produced during muscular exercise is transported to the adipose tissue where it is converted to fatty acids via pyruvate, the lactic dehydrogenase operating in the cycle. This would relieve some of the load on liver which has a variety of other functions to perform. It has so far been believed that muscle and liver are the only centres in which the conversion of lactic acid to other products takes place.

It should be of interest to note that when 2:3:5 triphenyl tetrazolium chloride was injected into the pigeon intravenously as well as intramuscularly the adipose tissue was found to be one of the tissues which showed the greatest dehydrogenase activity, the others being the kidney and the liver, as was evident from the deep red colour produced in the adipose tissues both subcutaneous and visceral. A detailed study of the role of adipose tissue in avian metabolism is in progress in our laboratories.

Summary

(1) A study of the histology, vascularity and certain enzymic activities of the visceral adipose tissue of pigeon was conducted.

(2) The tissue has a profuse supply of blood.

(3) Quantitative estimation of lipase showed a lipase activity of approximately 11μ moles of butyric acid/mg protein/hour.

(4) Histochemical study revealed a high activity of the following enzymes: lipase, alkaline phosphatase; succinic, malic, glycerophosphate and glutamic acid dehydrogenases.

(5) The significance of the high enzymic activity of the adipose tissue is discussed.

Acknowledgements

The DPN and 2:3:5 triphenyl tetrazolium chloride used in this study were supplied to us gratis by Sigma Chemical Company, U.S.A. and the Tween 80 by Atlas Powder Company, U. S. A. We are deeply indebted to them for their generosity.

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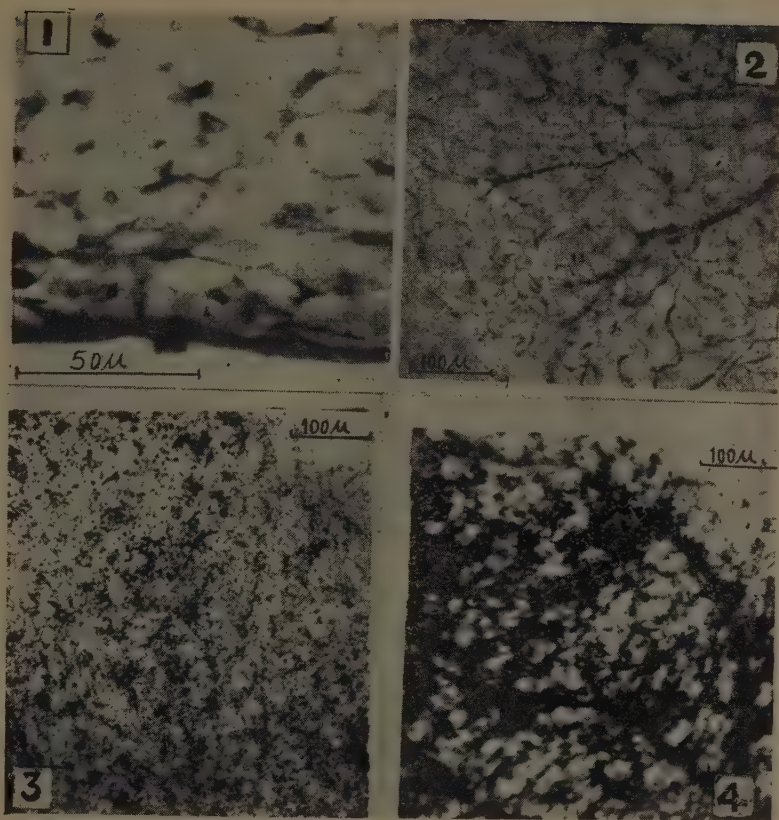


Plate 1

Explanation of figures.

Photomicrographs of sections of adipose tissue of the pigeon.

Figure 1: Section stained with haematoxylin and eosin to reveal histological details. The lower darkly stained portion is the thick collagenous covering, nuclei are located at the cell boundaries and the oval structures seen are the erythrocytes.

Figure 2: Section of adipose tissue injected with India ink, revealing capillary distribution.

Figure 3: Section treated to demonstrate lipase activity. Note the abundance of the precipitate deposited.

Figure 4: Section treated to show alkaline phosphatase activity. Capillaries as well as nuclei are deeply coloured.

STUDIES ON THE STRUCTURE AND PHYSIOLOGY OF THE FLIGHT MUSCLES OF BAT

2. The Relative Reduction of Fat and Glycogen in the *Pectoralis Major* Muscle During Sustained Activity

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CONSIDERABLE amount of reduction in the fat content of the *Pectoralis major* muscle and liver in some birds and a bat during prolonged exercise when the muscle is subjected to direct electrical stimulation, was reported by us in our earlier publication (1955). We now present data on the reduction of fat in the pectoralis muscle and liver in the bat reported earlier as well as the corresponding reduction in glycogen in these parts obtained in the same experiments, with a view to show as to which of the two energy fuels contributed the major part of the energy in such sustained muscular activity.

Material and Methods

The bat *Rousettus lechinaulti* was used in these experiments. The animal was pithed and pieces of the *pectoralis major* muscle from one side and the liver were cut out for the estimation of total lipid and glycogen. The intact muscle on the opposite side was electrically stimulated till it was completely fatigued and pieces of it and the liver were cut out for the estimations of total lipid and glycogen. All the methods employed, including those for the estimations of total lipid and glycogen were the same as in our earlier work (1955 and 1957).

Results

Since there exist considerable individual variations in the fat and glycogen contents of the muscle and liver depending on the physiological state of the animal, it was thought desirable to give the range in variation as well as the mean values in presenting the results. It is assumed that the reduction in the fat and glycogen contents of the muscle and liver at the end of the continuous muscular exercise denotes the amount of the substances utilized for energy. Reduction if any in the protein was very slight and was not taken into account. The respective energy values were obtained by multiplying the fat reduction figure by 9.5 and the glycogen reduction figure by 4.2 (Krogh and Lindhard, 1920).

TABLE I

Glycogen content and its reduction in the *pectoralis major* muscle and liver on the electrical stimulation of the former*

	Muscle		Liver	
	Average	Range	Average	Range
Percentage before stimulation	0.45	0.28-0.59	3.58	2.79-4.11
Percentage after stimulation	0.35	0.25-0.43	1.61	1.46-1.66
Actual reduction	0.10	0.03-0.16	1.97	1.33-2.45

* The values given are the mean of five experiments.

TABLE 2

Actual reduction in the total lipid and glycogen contents of the *pectoralis major* muscle and liver after electrical stimulation of the former with the respective and total energy values*

Total reduction of lipid per 100 gm. each of muscle and liver	1.88 gm.
Total energy value for lipid reduced in 100 gm. each of muscle and liver (L)	17.86 Cal.
Total reduction of glycogen per 100 gm. each of muscle and liver	2.07 gm.
Total energy value for glycogen reduced in 100 gm. each of muscle and liver (G)	8.69 Cal.
Total energy value for lipid and glycogen reduced in 100 gm. each of muscle and liver (L + G)	26.55 Cal.
Percentage of total energy obtained from lipid alone $\frac{L \times 100}{L+G}$	67.28
Percentage of total energy obtained from glycogen alone $\frac{G \times 100}{L+G}$	32.73

* The values for fat were taken from our earlier paper (1955).

Discussion

The *pectoralis major* muscle and liver of the pigeon contain 4.46% fat and 0.31% glycogen and 3.88% fat and 3% glycogen respectively, while those of the bat contain 12.32% fat and 0.45% glycogen and 6.84% fat and 3.6% glycogen respectively. The figures for fat are taken from George and Jyoti, (1955) and those for glycogen in the pigeon breast muscle and liver are from George and Jyoti (1957). It has been shown that after electrical stimulation of the breast muscle of the pigeon till it was fatigued, the reduction in the lipid and glycogen contents per 100 gm. of muscle as well as 100 gm. of liver corresponds to a total energy value of about 15 Calories calculated from the sum total of the amounts of fat and glycogen reduced in both muscle and liver together. Of these energy values again 71% of the energy utilized was obtained from fat alone while only the remaining from glycogen (George and Jyoti 1957).

From the results obtained from the experiments on the bat, it is seen that the total energy expenditure derived from fat and glycogen per 100 gm. each of muscle and liver amounts to 26.6 Calories of which 67.3% of the energy is obtained from fat and only the rest from glycogen (Table 2).

The conclusion to be drawn from this study is that the stored fuel in the flight muscle of the bat as in that of birds is to a great extent fat and that this material is the chief fuel in sustained muscular activity such as flight. This has also been shown in other flying animals like the desert locust (Weis-Fogh 1952). Therefore it seems evident that in the utilization of fat as the chief fuel during sustained muscular activity in the different flying animals, there is a physiological convergence. Apart from the muscle and liver as store houses of fat, fat is also stored as depot fat in other regions of the body which should be made available to the muscle when muscle fat gets depleted. This aspect is under investigation in our laboratories.

Summary

1. The *pectoralis major* muscle of the bat was exercised by direct electrical stimulation till the muscle was fatigued and the reduction in the fat and glycogen contents of the muscle and liver was noted.
2. From the results obtained it is found that fat is the chief fuel in such prolonged muscular exercise with about 67% of the total energy expended being obtained from this material and only the rest from glycogen.

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EFFECT OF COOKING BY DIFFERENT METHODS ON THE NUTRITIONAL VALUE OF FOODS*

Thiamine, Riboflavin, Nicotinic Acid, Vitamin A and Ascorbic
Acid Content of Cooked Foods

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THERE is a wide variation in the nutritional content of foods before and after cooking. Many of the articles of food are subjected to varying types of processes of cooking before they are eaten. The change in the chemical composition brought about by cooking has been a subject of study of very few observers. A large amount of work has been done on the Western Methods of cooking most of which merely consist of boiling, steaming or heating under pressure and so on. A great deal of work remains to be done on the influence of Indian methods of cooking on the nutritive values of foods.

The amount of work done in India on the influence of cooking on the nutritional value of foods prepared from cereals etc. like wheat and others is much too little compared to the detailed investigations made on rice, in spite of the fact that apart from rice, wheat etc. also form important cereals in Indian diets (Patwardhan, 1952). The chemical analysis of raw articles of food may sometimes be misleading as a guide to its nutritional value, because some of the material estimated in the raw articles may not be available for nutrition after they have been subjected to the varied changes during the process of cooking. The processes of cooking may differ from place to place and the duration of cooking may differ for different lengths of time. Some articles are cooked in a large quantity of water and the water is then thrown away. Most vegetables, for example, are boiled in large quantities of water before being eaten and the water is usually thrown away. On the other hand some articles are boiled in small quantities of water and this water retained. Some are

* Paper read at the Third Conference of Research workers in Gujarat held in Baroda in October 1957.

subjected to a high temperature with high pressure, while some to a low temperature. Thus we find that it has become almost a common procedure, especially in small middle class families where both husband and wife are office-goers, to use the ordinary cooker for cooking purposes, both from the economical point of view of fuel and time. On the other hand in the well-to-do families it is perhaps the common practice to use the pressure-cooker instead, which is rather a costly thing for the middle-class families to afford. Lastly the old traditional procedure, commonly followed by many people is by the direct application of heat to the utensils used for cooking.

A study was therefore undertaken to find out the differences in the retention of the water-soluble vitamins, namely thiamin, riboflavin, nicotinic acid and ascorbic acid in the cooked articles of food subjected to the above mentioned three different types of cooking procedures.

Material and Methods

The analyses of the samples with respect to the estimations of nicotinic acid, thiamine, riboflavin, ascorbic acid, and vitamins A were carried out in half the portion of the sample of the raw material and then in the remaining half after cooking by methods described in other communications made from here (Pai, 1954 and '57).

In case of these preparations, the condiments like chillies etc. were added before cooking; though the usual practice is to add them during cooking, because the uncooked preparation analysed was taken from the mixture made as above, while the remaining portion was cooked and then analysed. The procedure thus adopted gave more comparable data for the difference in the composition of uncooked and cooked items, from the point of view of studying the effect of cooking by these different procedures on the above nutrients of the foods.

In Table I the nutritive values of the food articles for their various constituents analysed are given on the basis of the dry weight in order to have a comparable data for each of the items in their uncooked forms on one hand, and cooked forms, on the other, which are subjected to the above mentioned different types of cooking procedures.

Pulses, both in the fresh and dried forms, *e.g.* rawan, peas, grams etc. were analysed in different stages namely raw, precooked and cooked.

In addition these very articles were cooked simultaneously by employing the above mentioned three different procedures, which were as follows :

- (1) Cooking by direct application of heat to the utensils.
- (2) Steam cooking in an ordinary cooker.
- (3) Steam cooking in a pressure cooker.

The following conditions for each cooking procedure were observed in order to have the controlled conditions all throughout the experimental procedures of cooking of the articles :

- (a) time required for complete cooking,
- (b) temperature of the cooked food preparation,
- (c) the quantity of water added to the preparation to aid cooking, etc.

The condiments *e. g.* chillies, salt, turmeric, etc. were added equally to both the preparations namely raw and pro-cooked in those cases where such raw articles were analysed after adding condiments to them. Each preparation was then made ready for analysis after making it homogenous by pounding it into a mortar with a pestle.

Preparations of the following items were analysed :

<i>Local Name</i>	<i>Botanical equivalent name</i>
(1) Rawan—	<i>Vigna catjana</i> Walp.
(2) Matki—	<i>Phaseolus aenitifolius</i> Tacquin.
(3) Peas (fresh)—	<i>Pisus arvene</i> Linn.
(4) Tur (fresh)—	<i>Cajanus indicus</i> Sprong.
(5) Gram (fresh)—	<i>Cicer ritinum</i> Linn.

Discussion

The results of analysis have been shown in the table. In the table the nutritive values of the food articles for their various constituents analysed are given on the basis of the dry weight in order to have a comparable data for each of the items in their precooked and cooked forms respectively as well as to study the effect of the three types of cooking procedures already described, on the retention of vitamins studied.

The previous results, already reported, had shown that the losses of the vitamins like thiamine, ascorbic acid, riboflavin and nicotinic acid are more in the preparations which are subjected to prolonged heating at high temperature than those in the cases of preparations which have been sub-

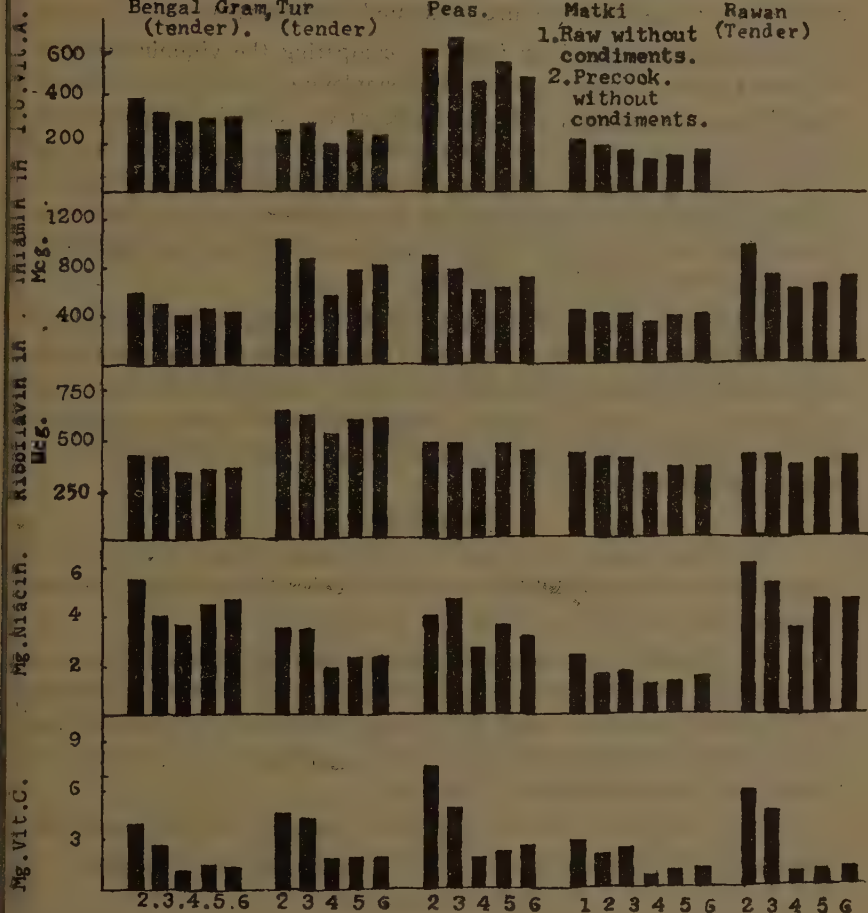
TABLE I
Thiamine, riboflavin, nicotinic acid, vitamin A and ascorbic acid content of cooked food preparation
per 100 grms. of dry weight

Sample No.	Preparation	Moisture Gms.	Vitamin A (I. U.)	Thiamine meg.	Riboflavin meg.	Nicotinic acid Mg.	Ascorbic acid Mg.
1	Peas (tender)	62.65	546.0	234.5	482.9	3.75	7.57
2	do do (precooked)	67.54	594.0	747.5	472.8	4.34	4.94
3	do cooked with direct heat . .	59.00	415.0	557.5	386.3	2.43	2.41
4	do steam cooked	66.80	483.0	636.5	462.4	3.36	2.53
5	do steam cooked with pressure . .	63.98	434.0	644.8	437.5	2.96	2.61
6	“Matki ” dry without condiments . .	7.40	199.2	436.6	379.2	2.34	2.79
7	do precooked without condiments . .	54.23	176.0	308.4	363.7	1.52	1.94
8	do precooked with condiments . .	47.89	168.8	384.7	359.9	1.65	2.22
9	do cooked with direct heat	54.42	114.6	336.1	228.8	1.19	0.70
10	do steam cooked	56.48	126.5	353.8	321.0	1.22	0.93
11	do do with pressure	57.49	152.0	370.1	323.4	1.44	0.96
12	“Tur ” (tender) raw with condiments . .	53.29	236.0	961.2	632.8	3.39	4.61
13	do precooked	58.64	266.0	830.3	610.5	3.38	4.17
14	do cooked with direct heat	54.62	132.0	498.3	505.4	1.84	1.48
15	do steam cooked	63.75	221.0	717.6	586.8	2.37	2.19
16	do steam cooked with pressure	63.78	212.0	781.6	574.6	2.32	1.93
17	Bengal gram (tender) raw	44.84	380.0	586.8	418.4	5.39	3.94
18	do do raw (precooked)	52.34	322.0	502.8	394.6	4.01	2.83
19	do cooked with direct heat	49.37	282.0	391.0	340.0	3.48	1.92
20	do steam cooked	57.12	298.0	442.0	363.8	4.36	1.69
21	do steam cooked with pressure	58.42	291.0	429.2	364.5	4.47	1.61
22	Rawan (Tender) raw with condiments . .	13.40	—	933.3	403.6	5.96	6.14
23	do precooked	46.65	—	690.1	395.3	4.98	4.48
24	do cooked with direct heat	50.65	—	562.6	342.4	3.34	1.30
25	do steam cooked	58.34	—	672.3	376.1	4.33	1.45
26	do steam cooked with pressure	55.08	—	644.8	367.3	4.28	1.36

2.Raw with condiments, 3.Precooked, 4.Direct heat,
5.Steam cooked, 6.Steam cooked with pressure.

Bengal Gram, Tur (tender). (tender) Peas. Matki Rawan (Tender)

1.Raw without condiments.
2.Precook. without condiments.



Effect of cooking by different methods on the Thiamine, Riboflavin, Niacin, Vit. C, and Vit. A contents of foods (on dry weight basis).

jected to heating at low temperature for a short interval of time. These findings have been confirmed here by the present results obtained in case of the above preparations studied (vide table and charts).

Furthermore it has been found on comparing the vitamin contents of cooked food articles prepared after employing the above mentioned three different procedures, that the losses of the vitamins studied, were more in the first type, *i. e.* ordinary cooking by direct application of heat to the cooking utensils than those in the cases of either of the other two cooking procedures *i.e.* steam cooking in an ordinary cooker and steam cooking in pressure cooker (vide table and charts). These findings are in conformity with those of other workers.

Cruikshank (1951) stated that pressure cooking is not particularly much destructive of thiamine. Chappell and Hamilton (1949) have made a statement, in view of the findings made from several investigations, that with respect to carotene, thiamine, nicotinic acid and ascorbic acid, their retention in pressure cooking is not likely to be worse but probably better than in open methods.

Conclusions

(1) The results of analysis of twenty-six items of cooked food preparations studied for their thiamine, riboflavin, nicotinic acid, vitamin A and ascorbic acid content, have been presented.

(2) Losses of the vitamins—ascorbic acid, thiamine, riboflavin and nicotinic acid are comparatively more during cooking by direct application of heat to the containers used for cooking than when the cooking was done either in ordinary cooker (by steam cooking) or in the pressure cooker (by steam-cooking with pressure).

(3) The results obtained have been compared with the findings of other workers and the significance of the findings have been discussed.

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STUDY OF UREA CLEARANCE TEST IN NORMALS

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TESTS based on a measurement of the capacity of the kidney to clear waste products of metabolism, such as urea, creatinine etc. from the blood in a given time, have been included in the group of kidney function tests. Only a one-sided picture would be obtained if the analysis of either the blood alone or urine alone is carried out for the urea content. More complete information will be available by comparing urine and blood composition at the same time, such as in the case of tests known as "clearance tests" (Herrin, 1941; Beaumont & Dodds, 1944). One of these tests which is very often used in the clinical laboratory is "blood urea clearance test" (Möller, McIntosh & Van Slyke, 1929). This test has been reported after a study on 18 normal subjects, to yield results bearing fairly close correspondence with the clinical findings in patients. The standard for normal clearance has been based on the above observations on American subjects. The average values given by the authors were 54 cc. for standard clearance (Cs) and 75 cc. for maximum clearance for a mean body surface area of 1.73 sq. m. Since then, these above-mentioned standards have been adopted for assessing the urea clearance in health and disease in other countries as well.

Investigations on urea clearance test carried out on 110 subjects from Bombay led to the conclusion that the average urea clearance either maximum or standard in normal Indians was only 60 per cent of the American standard (Gokhale, 1941). According to the findings of the author, the average standard clearance is 31.5 cc. and the maximum clearance is 40.01 cc. without correction for the body surface area and 33.8 cc. and 44.0 cc. respectively after correction for the body surface area of 1.73 sq. m. Shrinkantia and Shamanna (1944) studied 57 Mysoreans for their urea clearance test and their figures for the average standard clearance were 35.95 and for the maximum clearance 46.83 cc. without the correction for body surface area and 39.36 and 52.26 cc. respectively after correction.

Pai (1945) reported a study of non-protein nitrogen contents of

serum and of blood urea clearance test in 32 patients most of whom were admitted for malignant disease but none of them had a kidney tumour or any clinical evidence of kidney dysfunction. In these patients the standard clearance ranged from 16.60 to 51.30 cc. with an average of 35.16 cc. These values appeared to indicate an impaired kidney function on the basis of American standards but normal function on the basis of Indian values.

It was remarked in Nature (1945) that "the figures of Gokhale and of Shrinkantia and Shamanna suggest that the Indian kidney has only about two-thirds the efficiency of its American counterpart; but whether this is true or not, it is obvious that a new and lower normal standard will have to be adopted for clinical work on Indians".

Very few studies have since then been made to find out the appropriate standards with regard to the blood urea clearance tests, as stated above, for Indians. For such a study, investigation on normal subjects belonging to different age-groups and different parts of India, will have to be undertaken. It was therefore thought worthwhile to carry out such a study on some normal subjects belonging to this part of the country, namely Gujarat, the results of which have been presented here.

Material and Methods

Eighteen normal male subjects with age varying between 17 and 22 years have been studied for the blood urea clearance test. The technique of the method used in this series was the same as that described by Möller *et al* (1929) and the same as that followed in the earlier communication (Pai, 1945). The subject came in the morning after a 12 hours fasting period, and was asked to evacuate the bladder completely before starting the test. A glassful of water was given to drink and the correct time noted. Blood sample was with drawn by a vein-puncture at 50 minutes interval from the above time and two urine samples were collected exactly at the end of one hour and the two hours interval respectively from the above time. Care was taken to see that at each time of the collection of the urine, the bladder was completely evacuated. The blood and the urine samples were then analysed for their urea content and for other data, the results of which are shown in table I.

Discussion

Out of the eighteen normal subjects studied, eleven subjects had maximum clearance and the remaining *i. e.* seven subjects had standard

TABLE I
Blood Urea Clearance in Normal Subjects

Subject	Age in years	Height in inches	Weight in lb.	Body surface area sq. meters (B.S.A.)	Volume of urine cc.		Urine urea mg. %			Blood urea mg. % (B)	Urea clearance corrected for B.S.A. cc.	
					1st hr.	2nd hr.	Aver/mt (V)	1st hr.	2nd hr.		Max. Cm. = $\frac{U \times V}{B}$	Standard Cs = $\frac{U \sqrt{V}}{B}$
1 P.P.	20	64.6	103	1.46	350	113	3.54	175	350	34.0	39.4	—
2 J.V.	19	66.0	127	1.76	274	140	3.48	320	460	21.0	64.3	—
3 D.T.	19	69.0	134	1.73	279	136	3.49	770	430	33.0	61.4	—
4 P.F.	20	62.2	108	1.45	168	178	2.9	500	400	26.0	59.5	—
5 C.P.	17	67.7	117	1.61	578	148	6.0	100	600	29.6	74.0	—
6 T.C.	20	66.5	120½	1.61	130	126	1.92	550	400	21.0	—	34.1
7 B.R.	18	70.0	146	1.81	128	114	1.93	650	500	22.0	—	35.0
8 N.S.	18	64.0	118	1.59	243	234	3.56	110	125	20.0	27.6	—
9 S.S.	19	66.0	116½	1.58	74	213	2.1	1000	350	34.0	45.3	—
10 N.M.	21	67.4	111	1.58	68	92	1.2	1380	890	22.0	—	62.1
11 S.J.	22	64.5	109½	1.52	176	245	3.5	450	280	36.0	40.4	—
12 B.D.	18	66.0	106	1.52	200	256	3.8	420	220	20.0	69.0	—
13 D.P.	18	63.5	108	1.50	100	289	3.6	620	200	24.0	68.8	—
14 J.A.	17	65.1	105½	1.50	135	79	1.8	230	550	20.0	—	28.3
15 D.R.	18	63.5	107½	1.50	137	78	1.7	980	1100	32.0	—	48.6
16 K.B.	20	65.4	120½	1.60	165	53	1.81	650	780	30.0	—	34.5
17 S.C.	21	68.0	109½	1.58	45	17	0.52	1650	1500	20.0	—	62.1
18 P.S.	20	63.0	139	1.65	151	88	2.0	620	760	30.0	48.2	—

clearance (table I). These subjects were given a clinical overhaul and they were accepted for this investigation only on satisfying the conditions that they neither suffered from any illness at the time nor had they any history of previous illness connected with kidney dysfunction. The diet of these subjects were mostly the vegetarian type in which milk in moderate quantity was included. Some of them had no objection to taking eggs but took only on very rare occasions. On analysis of data collected of their dietary intake, it was found that on the average it was a low protein diet as compared to the protein content of the meat diet taken by the non-vegetarians of the Western countries.

In this series, the values for the standard clearance varied between 28.3 to 62.1 cc. of blood cleared of urea per minute. The average standard clearance was 43.3 cc. The range for the maximum clearance was 27.6 to 74.0 cc. with the average value of 54.3 cc. Lately the results of a similar study have been reported by Chitre (1953). This study was made on hundred clinically normal subjects, who were mostly medical staff and students ranging between the ages of 20 and 38 years. Out of these, 49 subjects subsisted on mixed dietary, while the rest were strictly vegetarian. The range for maximum clearance was found to be 40 to 59.5 cc. with an average of 45.6 cc., whereas the standard clearance ranged from 31.5 to 47.0 cc. with the average value of 36.6 cc. These values are a little lower than those obtained in the present series. But as can be seen from the table, all the values that have been given in the preceding paragraphs which have been reported after the study on Indians are definitely lower, both for the standard clearance and the maximum clearance, than the corresponding values obtained in the West (table II).

TABLE II
Comparison of Standard and Maximum Clearance

Series	Mean standard clearance c.c.	Mean maximum clearance c.c.
Möller (1929)	54.0*	75.0*
Gokhale (1941)	33.8*	44.0*
Shrikantia and others (1943)	39.4*	52.3*
Shamanna (1944)		
Pai (1945)	35.2	—
Chitre (1953)	36.6	45.6
Present series	43.3*	54.3*

* Corrected for body surface area of 1.73 sq.m.

The urinary urea in the subjects studied, when expressed in mg. % (*i.e.* U) varied between 117 and 1575 with the mean value of 584; whereas the blood urea (*i.e.* B) ranged between 20 and 36 mg. % with an average value of 26.4 mg. % (table I). Gokhale (1941) had obtained 454 mg. % and 26.3 mg. % as the average values for the urinary urea and blood urea respectively. Here again the findings in the present series are therefore in agreement with those of the other workers, and they once more show that the blood urea clearance in normal Indians is lower than in the normal subjects of Western countries. Secondly the fact that the blood urea in Indians is more or less the same as in Westerners, whereas the urinary urea of the former is lower than that of the latter which may be due to the lower dietary protein intake of Indians and/or different vegetarian composition of Indian dietaries, may be the responsible factor for their lower urea clearance.

Summary and Conclusions

(1) Eighteen normal male healthy subjects of age varying between 17 and 22 years have been studied for their blood urea clearance test.

(2) The standard clearance in seven of the above subjects ranged from 28.3 to 62.1 cc. with an average value of 43.3 cc; whereas the maximum clearance in the remaining eleven subjects varied between 27.6 and 74.0 cc. with the mean value of 54.3 cc.

(3) The urinary urea ranged from 117 to 1575 mg.% with an average of 584 mg.%; whereas the blood urea varied from 20 to 36 mg.% with the mean value of 26.4%.

(4) The blood urea clearance, both standard and maximum, is lower in normal Indians as compared with that of normal Westerners.

(5) The fact that blood urea is more or less the same in both Indians and Westerners, whereas the urinary urea in Indians is lower than in Westerners, which may be due to lower dietary protein intake of Indians and/or different vegetarian composition of Indian dietaries, may be responsible for their lower urea clearance.

(6) The above findings have been compared with those of other workers and their significance has been discussed.

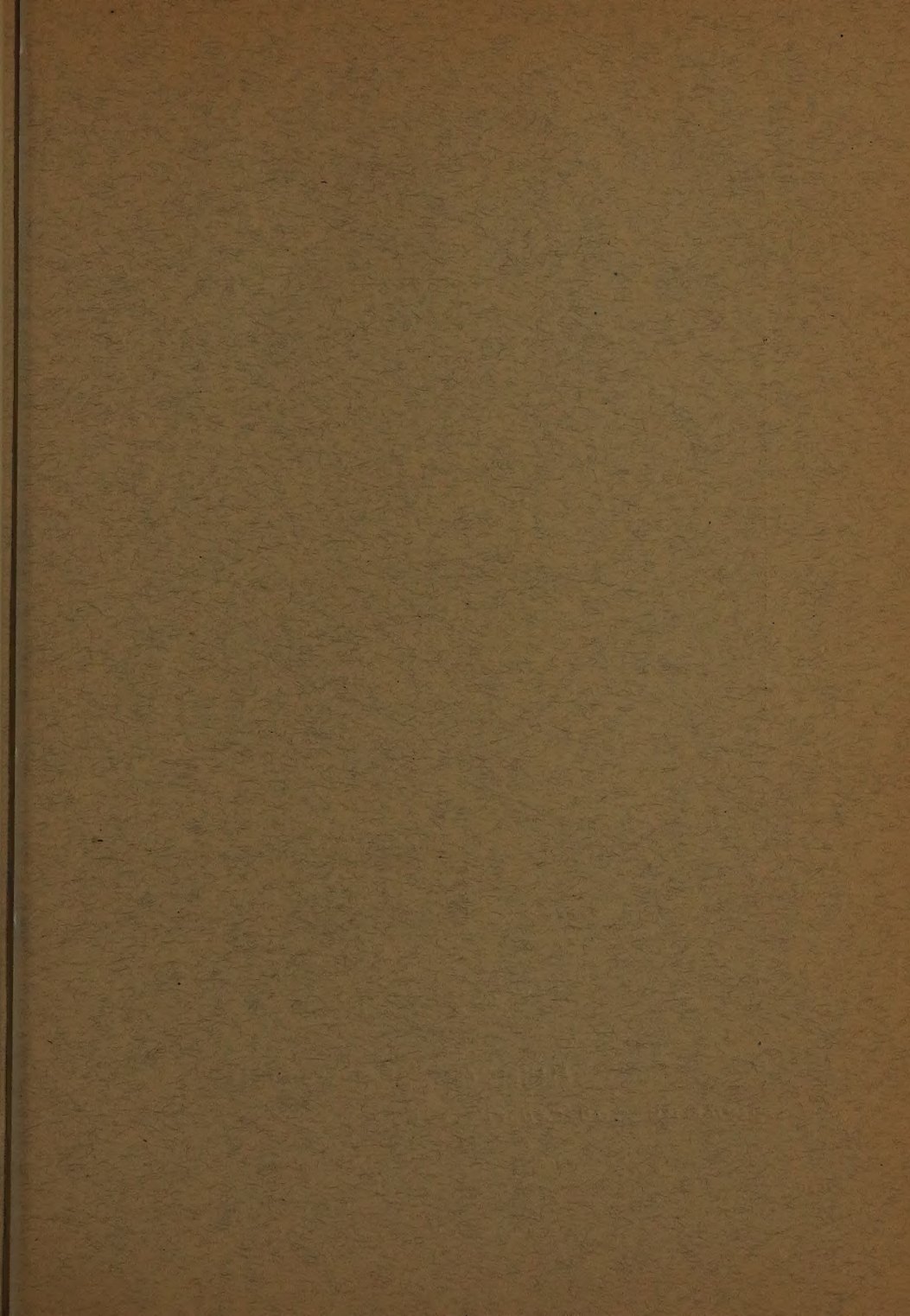
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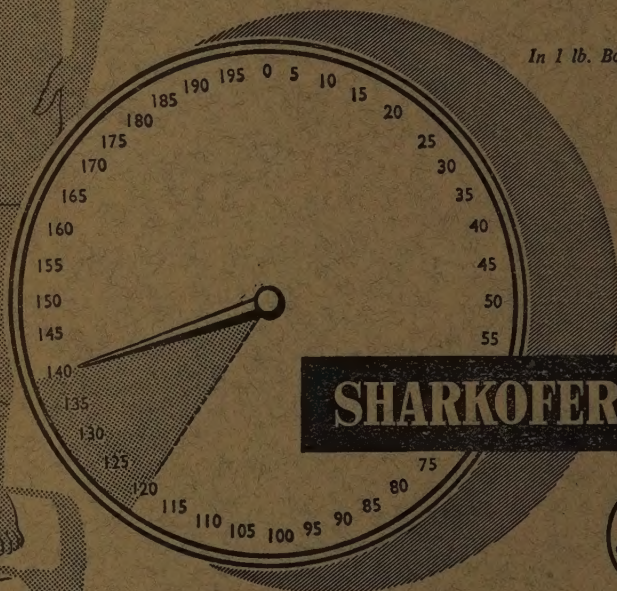
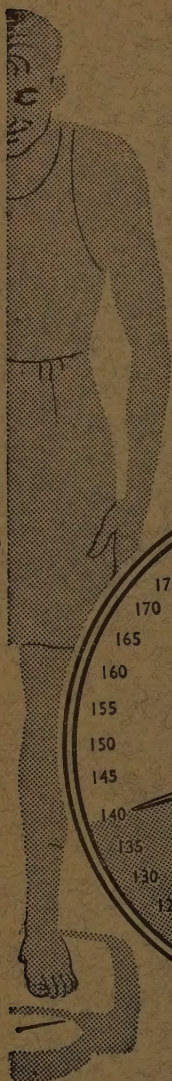
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